The relation between initial corneal hydration and stiffening effects of collagen crosslinking treatment

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Short Title: Biomechanics of preswollen collagen crosslinked cornea

- This project has been funded in whole or in part with fund from National Science Foundations.
- The authors have no financial or proprietary interest in a product, method, or material described herein.

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Abstract: (250 words)

Purpose: To characterize the mechanical property improvement of riboflavin/UV corneal collagen crosslinking procedure in samples that are artificially swollen prior to being treated.

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

Design: Experimental study

Methods: Both porcine and human donor corneas were collagen crosslinked at different hydration levels using riboflavin/dextran solutions of different osmolality. Four hydration groups (H_w =3.3±0.2, 4.0±0.1, 5.1±0.1, and 5.6±0.1 *mg H_2O/mg dry tissue*) were considered for porcine samples and three hydration groups (H_w = 3.2±0.1, 3.9±0.2, 5.3±0.3 *mg H_2O/mg dry tissue*) for human samples. 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. Additional porcine samples were crosslinked at average hydration of 5.1 and 5.6 *mg H_2O/mg dry tissue* and their tensile properties were measured when their hydration was lowered to 4.0 *mg H_2O/mg dry tissue*.

Results: The collagen crosslinking procedure significantly increased the tensile properties of both human and porcine samples in each hydration group ($P<0.05$). The improvement in tensile properties was hydration-dependent, i.e. samples that were crosslinked at higher hydration levels had lower maximum tensile stress. The behavior of samples crosslinked at different initial hydration but tested mechanically at similar hydration showed no significant difference ($P=0.7$).

Conclusions: Increasing the hydration of porcine and human corneal samples prior to the collagen crosslinking treatment had insignificant influence on tensile property improvement, as measured by testing the specimens at similar hydration.

Keywords: Uniaxial tensile experiments, pre-swollen, crosslinking, biomechanics, porcine and human cornea

Introduction

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 which 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. Though the exact underlying cause of keratoconus has not yet been determined, it is associated with genetic disorders such as familial inheritance, Down's syndrome, and environmental factors such as excessive eye rubbing and hard contact lens wear [1-3].

The riboflavin/UV collagen crosslinking (CXL) is a clinical treatment procedure, which improves the mechanical strength of keratoconus cornea. Over the past decade, the effectiveness of crosslinking treatment in stiffening the cornea and halting the keratoconus has been established by different in vitro and in vivo studies [4-10]. The common collagen crosslinking procedure, often known as the Dresden protocol, has two steps. The first step involves removing the corneal epithelium and soaking the stromal layer in a photosensitizer solution made up of 0.1% riboflavin and 20% dextran. 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 with advanced keratoconus, whose corneal thickness is less than 400 um, because UV irradiation will damage 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 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 collagen crosslinking 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 collagen crosslinked corneas and compared the results with the mechanical properties of untreated samples. We used both human donor and porcine cadaver corneas in order to characterize possible differences in the mechanical behavior of the cornea from different species.

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 a day. Human donor corneas had been stored in Optisol solution for about one month post-mortem before they were brought to the laboratory. In order to crosslink specimens at different hydration levels, we used sample thickness as a surrogate for their hydration. The thickness-hydration relations of $H_w = 7.0\ t - 0.64$ and $H_w = 3\ln(t/0.2)$ 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 immersing in saline solution in order to adjust their initial hydration before being crosslinked.

Five samples in each thickness group were crosslinked and five specimens were used as control. The photosensitizer solution was prepared using riboflavin and dextran T-500. 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 mm wide strips were punched in the nasal-temporal direction using a custom built double bladed punch. Strips dissected from nasal–temporal direction were only used in order to prevent variations in the experimental measurements due to the corneal anisotropic response [14, 15]. The strips were exposed to UV rays of intensity 3mW/cm^2 at about 1-2 cm distance for 30 minutes, while few drops of the photosensitizer solution were added every 5 minutes, Figure 1. The irradiance was measured using a radiometer (Solarmeter, Pennsylvania). The crosslinking 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. These samples were referred to as control 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 crosslinking treatment. Thus, a pilot swelling study was performed to determine the appropriate concentration of dextran T-500 (Sigma-Aldrich Co. LLC) in the photosensitizer riboflavin/dextran solution such that unwanted hydration changes due to deturgescent effects of dextran can be avoided. The objective of this preliminary study was to find the required dextran concentration for which the thickness (hydration) remained almost constant when the samples were immersed in the photosensitizer solution. Riboflavin/dextran solutions of different osmolality were made by varying the concentration of dextran between from 2.5% to 20%. 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 time intervals using a digital pachymeter (DGH Pachette 3, DGH Technology Inc., Pennsylvania). Using this study, we used dextran concentration of 20%, 10%, 5%, and 2.5% for porcine samples and divided them into four groups. Due to the limited number of available human donor tissue, we did not do this pilot swelling study on these samples and used dextran concentrations of 20%, 15%, and 10% in order to divide the samples into three groups. Table 1 gives the average thickness, hydration, and dextran concentration of porcine and human specimens.

Before starting the mechanical tests, the thickness of treated and the control samples was measured using a digital pachymeter, and the width of strips was measured using a digital calliper (Mitutoyo Corp. Japan). A DMA machine (RSA-G2, TA Instruments, Delaware, USA) was used to perform uniaxial tensile tests and measure the biomechanical properties of the strips. Sand papers were used to ensure that there was no slippage. The strips were mounted at a loading gap of about 7 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 2mm/min. The tensile stress was obtained by 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 coefficients of determinations R^2. Furthermore, the tangent modulus and the tensile stress at 6%, 8%, and 10%

strain were calculated and reported as mean ± standard deviation. The maximum tensile, σ_{max}, is defined as the stress at 10% strain, ε_{max}=10%. One-way ANOVA with a p-value of 0.05 was performed in order to determine the significant difference between the mechanical behaviour of various groups.

Results

Figures 2-3 shows the stress-strain behavior of crosslinked and control porcine and human samples. It is seen that with increasing the hydration (thickness), the mechanical response of both human and porcine specimens became softer. The numerical values of the maximum tensile stress for control porcine and human samples is given in Table 1. The difference between various hydration porcine groups was significant ($P<0.05$).. Furthermore, except for group H1 and group H2 ($P=0.15$), the difference between individual human groups was significant ($P<0.05$). Furthermore, samples which were crosslinked using more hypotonic riboflavin solution had a lower peak tensile stress compared to those treated with less hypotonic or isoosmolar solution. The maximum tensile stress for crosslinked porcine and human samples is also given in Table 1. The difference between various hydration porcine groups was significant ($P<0.05$). For crosslinked human samples, except for group H1 and group H2 ($P=0.2$), the difference between all other individual groups was significant ($P<0.05$). Comparing the tensile stress for crosslinked and control samples within each hydration group showed that the collagen crosslinking procedure significantly improved biomechanical properties of the cornea ($P<0.05$). Finally, comparing the maximum tensile stress of human and porcine groups of almost similar hydration showed that human samples had significantly stronger tensile properties that those of the porcine

strips ($P<0.05$). Table 1-2 give the tangent modulus and tensile stress of different groups at 6%, 8%, and 10% strain. The exponential mathematical function was able to successfully curve-fit the stress-strain curves of all porcine and human hydration groups, the solid lines in Figures 2-3 represent the numerical fits. The fitting parameters and the coefficients of determination are given in Table 2.

Discussion

In the corneal crosslinking treatment method, the corneal tissue is first soaked in the photosensitizer solution and is then subjected to UV rays. The soaking step is included to ensure sufficient penetration of riboflavin into the corneal stroma. Riboflavin, by production of singlet oxygen molecules upon exposure to UV rays, enables creation of crosslinks.

In the corneal crosslinking treatment procedure, the thickness of the cornea should not be less than 400μm in order to avoid damage to the endothelial cells because of UV radiation [8, 17]. Avedro, Inc., which received FDA approval in 2016 for its crosslinking device called KXL system, lists that in cases where the thickness of the cornea goes below 400μm after the soaking period, 0.1% riboflavin solution without dextran needs to be used. This process is expected to cause corneal swelling and increase the thickness to the required minimum of 400 μm.

The corneal extracellular matrix is primarily composed of collagen fibrils and proteoglycans [18]. The proteoglycans consist of negatively charged glycosaminoglycans, which are ionized at physiological pH. Thus, a net negative fixed charge density exists inside the stroma. Because of these negative charges, the cornea could swell significantly when immersed in an ionic solution [12, 13]. In the common crosslinking protocol, dextran is used to prevent corneal swelling and keep

its thickness unchanged. In the modified collagen crosslinking procedure, dextran is not added to the riboflavin solution in order to allow free swelling of the cornea.

In a recent study, the modified CXL procedure was performed on twenty patients whose corneal thickness was between 320 um and 400 um and no complication was reported [11]. There were other clinical studies in which hypotonic riboflavin solutions were used to treat thin corneas with success [19-21]. However, the important question that yet remains unanswered in the field is that whether the stiffness of the crosslinked swollen corneas is different than the stiffness of the corneas which are crosslinked at their normal thickness. This is an important question because it has been shown that corneal mechanical properties are a function of hydration [9, 16, 22].

The primary objective of the current study was to characterize how artificially swelling the cornea before collagen crosslinking would affect the improvement of tensile properties due to this procedure. To this end, we conducted mechanical experiments on both human and porcine corneas, which were crosslinked at different initial hydration levels, and compared their mechanical response to the behavior of control samples with similar hydration.

Consistent with previous studies on bovine corneas [16, 22], Figures 2-3 show an inverse relation between hydration level and the tensile strength in porcine and human cornea. Furthermore, the maximum tensile stress of crosslinked strips was significantly higher than the maximum tensile stress of control strips with the same hydration, Table 1. The samples in the control groups were subjected to the same testing conditions as their respective crosslinked strips with the exception of being exposed to UV rays. Consistent with previous reports [6], Figure 2 shows that the collagen crosslinking treatment increased corneal tensile stress at 6% by about 70%. Additionally, it is observed that the increase in tensile properties is hydration-dependent and depends on the hydration at which the samples are crosslinked. For instance, at 6% strain level, the increase in

tensile stress was equal to about 77%, 51%, 38% and 16 % for porcine samples crosslinked at average hydration of 3.3, 4.0, 5.1, and 5.6 mg water/mg dry tissue, respectively. Furthermore, in human donor samples, we observed an increase of 58%, 55% and 30% increase in the tensile stress at 6% strain in hydration groups 3.2, 3.9 and 5.3 *mg H_2O/mg dry tissue* respectively. In the following, we attempt to explain this behavior in terms of the inhomogeneous microstructure and swelling properties of the corneal extracellular matrix as well as the depth dependence of corneal stiffening due to the collagen crosslinking procedure.

It has been shown that anterior collagen lamellae in the corneal stroma interweave while the posterior lamellae are almost parallel to each other [18, 23, 24]. Previous studies have also shown that the crosslinking procedure improves significantly the mechanical properties of the anterior 200 um of the cornea and it has a lesser effect on biomechanics of the remaining posterior layer. Kohlhaas et al. crosslinked porcine cornea using the Dresden protocol and measured the tensile properties of anterior 200 um flaps dissected from the crosslinked samples [25]. They observed that the stiffening effect was much more significant in these anterior flaps. This observation was attributed to the fact that about 65%-70% of UV radiations is absorbed in the anterior 200 μm and the rest is absorbed in the posterior region. It is also known that the swelling behavior of cornea is inhomogeneous and the posterior stroma swells more than the anterior stroma [26, 27]. This behavior is because collagen lamellae in the anterior part are tightly interwoven, and therefore, are resistant to swelling. Thus, we believe that the stiffening effect of the CXL procedure is hydration dependent because the ratio of the crosslinked thickness and the total thickness is decreased with increasing the hydration, Figure 4. Also note that, as shown in previous studies, mechanical properties of the cornea become softer with increasing thickness (hydration) because the bonds between the collagen fibrils and proteoglycans break [16, 22].

Considering the above discussion and the experimental results of the present work, we hypothesize that increasing the hydration of samples prior to collagen crosslinking does not significantly affect the portion of the cornea that is crosslinked, Figure 4. If this hypothesis is true, the observed difference in tensile properties of specimens crosslinked at different hydration (thickness) should be mainly because of hydration-dependent mechanical properties of the cornea [16, 22, 28]. To test this hypothesis, we crosslinked five porcine corneal strips with the average initial hydration of 5.1 *mg H_2O/mg dry tissue* and another five at H_w=5.6 *mg H_2O/mg dry tissue*, air dried them until their hydration reached 4.0 *mg H_2O/mg dry tissue* (hydration of group P2), and then measured their tensile properties. We observed that the tensile behavior of these specimens matched those of samples which were crosslinked at initial hydration of 4.0 mg H_2O/mg dry tissue, Figure 5. This finding confirms that the effectiveness of crosslinking treatment does not significantly change if the samples are pre-swollen. Nevertheless, future studies are required to test this hypothesis in vivo and especially on keratoconus corneas. Furthermore, future imaging studies are required to determine the effective depth of collagen crosslinking in artificially swollen samples. Also, crosslinking using hypoosmoloar solution may have important effects on other aspects of corneal properties, e.g. viscoelasticity, that have not been considered in the present work.

At the same hydration levels, human donor corneas showed a stiffer tensile response compared to porcine corneas. For instance, at hydration of about 3.2-3.3 *mg H_2O / mg dry tissue*, the tangent modulus at 6% strain for control and crosslinked human samples was 5.6 ± 1.5 MPa and 3.2 ± 0.7 MPa while it was 2.4±0.3 MPa and 1.4±0.2 MPa in control and crosslinked porcine samples, respectively. The stronger tensile behaviour of human donor tissue in comparison to porcine cornea is in agreement with previous studies [6, 29]. Nevertheless, it is noted that the

mechanical properties of cornea vary with age and hydration [28, 30]. Although this work characterized the tensile behaviour of human cornea as a function of hydration, it did not characterize the effects of age of samples on their mechanical properties because their age was not known. Another limitation is that the donor samples were in optisol for one month prior to being used in this study.

In our previous work on hydration-dependent properties of the cornea and some other related work in the literature, samples were preconditioned prior to the mechanical tests [9, 14, 22, 29]. However, the specimens were not preconditioned here to reduce the time of experiments. In order to characterize the effect of preconditioning procedure, we tested additional porcine samples with an initial preconditioning step [14]. Five untreated and five crosslinked samples with average hydration of 3.3 *mg H_2O/ mg dry tissue* were used. The preconditioning included five loading/unloading cycles upto 10% strain and three relaxation steps. Figure 6 compares the stress-strain behavior of preconditioned and not preconditioned samples. The CXL treatment increased tensile stress of preconditioned and not preconditioned samples by about 70% and 77%, respectively. The results shown in Figure 6 imply that similar conclusions would have been reached if a preconditioning step had been used to measure the tensile properties of artificially swollen crosslinked cornea.

Furthermore, since the duration of the experiments in the current study was short, no bathing solution was used. However, oil has often been used as the bathing solution in order to ensure that the hydration (thickness) of samples remains constant during the mechanical tests [9, 14]. We tested a group of crosslinked human samples in oil in order to show that using a bathing solution was not required in this study, Figure 7. No significant dehydration would occur between the

end of crosslinking and the end of the tensile tests; this was confirmed by measuring the specimen thickness at the end of mechanical tests.

Finally, in an effort to estimate the tensile behavior of corneal samples with arbitrary hydration from the stress-strain behavior of corneal samples with known hydration, we plotted the normalized stress $\bar{\sigma} = (\sigma - \sigma_0)/(\sigma_{\max} - \sigma_0)$ and normalized strain $\bar{\varepsilon} = \varepsilon / \varepsilon_{\max}$ for the results shown in Figures 2-3. Here, σ_{max} is maximum stress at ε_{max}=10%. We observed that the stress-strain data for different hydration groups in each figure collapsed onto a universal curve represented by the equation $\bar{\sigma} = (e^{\bar{B}\bar{\varepsilon}} - 1)/(e^{\bar{B}} - 1)$ where $\bar{B} \simeq 2.07$ and 2.17 for pseudo-crosslinked and crosslinked porcine samples (Figure 2), and $\bar{B} \simeq 2.27$, and 2.61 for pseudo-crosslinked and crosslinked human samples (Figure 3), respectively. It is noted that the present work shows that these universal curves at least exists for the range of hydration considered here; its presence outside this range needs future studies. However, we expect that the lower limit to be around hydration 1-1.5 mg *mg H_2O/mg dry tissue* where the variation in hydration has been reported to cause significant changes to the ultrastructure of the cornea [31]. The underlying mechanisms for this interesting experimental observation need to be investigated in future works.

In summary, this work characterized the improvement in mechanical properties of human and porcine corneas due to the riboflavin/UV collagen crosslinking treatment as a function of hydration. The concentration of dextran (a deturgescent agent) in riboflavin solution was varied to control the hydration (thickness) prior to the collagen crosslinking treatment. The improvement in tensile properties due to the collagen crosslinking treatment was significant in all groups ($P<0.05$) but it was inversely proportional to the initial hydration of the specimens. Furthermore, the tensile behavior of specimens which were treated at different hydration but

tested at similar hydration was similar. This suggests that hydration-dependent biomechanical properties of the cornea should be why a significant difference was observed in tensile properties of specimens that were crosslinked at different hydration. Future studies are required to check whether collagen crosslinked corneas using isoosmolar and hypoosmolar riboflavin solutions show similar response in vivo conditions.

Acknowledgements:

The authors acknowledge support for this work in part from NSF-CMMI-1635290

WHAT WAS KNOWN:

The riboflavin/UV collagen crosslinking treatment improves the biomechanical properties of the cornea. In order to avoid toxic effects of UV irradiation on corneal endothelial cells, the corneal stroma should be at least 400 um thick. In patients with thin corneas, a hypoosmolar riboflavin solution can be used to swell preoperatively the cornea to a thickness of larger than 400 um. Nevertheless, considering the hydration-dependent biomechanical properties of the cornea, possible influence of the above procedure on stiffening effects of collagen crosslinking procedure is not known.

WHAT THIS PAPER ADDS:

This paper showed that the tensile stiffening effect of collagen crosslinking treatment was dependent on the initial hydration of corneal samples. In particular, crosslinking the specimens at higher initial water content resulted in lower improvement in their tensile stress and tangent modulus. Nevertheless, the tensile properties of corneas treated at different hydration but tested mechanically at similar hydration was similar. Thus, corneal pre-swelling had an insignificant effect on the tensile property improvement caused by collagen crosslinking method; the difference is due to corneal hydration-dependent mechanics.

Figure and Table Captions:

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

Figure 2. The tensile stress-strain behavior of a) control and b) crosslinked porcine samples. The hydration and thickness of different groups are given in Table 1. The solid lines represent the numerical exponential fits. The fit parameters are given in Table 4. Hydration of groups P1, P2, P3, and P4 is H_w =3.3±0.2, 4.0±0.1, 5.1±0.1, and 5.6±0.1 *mg H_2O/mg dry tissue*, respectively.

Figure 3. The tensile stress-strain behavior of a) control and b) crosslinked human samples. The hydration and thickness of different groups are given in Table 1. The solid lines represent the numerical exponential fits. The fit parameters are given in Table 4. Hydration of groups H1, H2, and H3 is H_w = 3.2±0.1, 3.9±0.2, 5.3±0.3 *mg H_2O/mg dry tissue*, respectively.

Figure 4. A schematic plot that describes the possible reason for the experimentally measured hydration dependent stiffening of the collagen crosslinking procedure. a) The microstructure of the cornea is anisotropic through the thickness: h and h_a denote the thickness of whole and anterior part of the cornea, respectively. b) When the cornea is swollen, the amount of swelling in not uniform and the anterior region swells less compared to the posterior part: h' and h'_a denote the thickness of whole and anterior part of the swollen cornea, respectively. c) The collagen crosslinking affects primarily the top layers of the cornea, denoted by h_{cxl} and red vertical crosslinks. Thus, the crosslinking is limited to the anterior layers. d) When the corneal hydration is reduced to the normal amount, the effective crosslinking depth is almost remains unchanged and as if the sample was crosslinked at its normal hydration.

Figure 5. The tensile stress and tangent modulus at 6% strain of porcine strips crosslinked at different hydration level but all tested at the same average hydration (H_w ~4.0 mg H_2O/mg dry tissue) of the control group. It is seen that amount of stiffening is independent of the initial hydration of specimens when they are crosslinked.

Figure 6. The effect of preconditioning procedure on the tensile stress and tangent modulus at 6% strain for control and collagen crosslinked porcine cornea.

Figure 7. The effect of bathing fluid on tensile stress and tangent modulus at 6% strain of human corneas.

Table 1. Dextran concentration, thickness, and hydration, and tensile stress in KPa at 6%, 8%, and 10% strain for control and treated porcine and human corneal strips..

Table 2. Tangent modulus in MPa at 6%, 8%, and 10% strain, and the fit parameters for control and treated porcine and human corneal strips. The coefficients of determination were larger than 99% for all groups. The hydration and thickness of different groups are given in Table 1.

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Figure 1

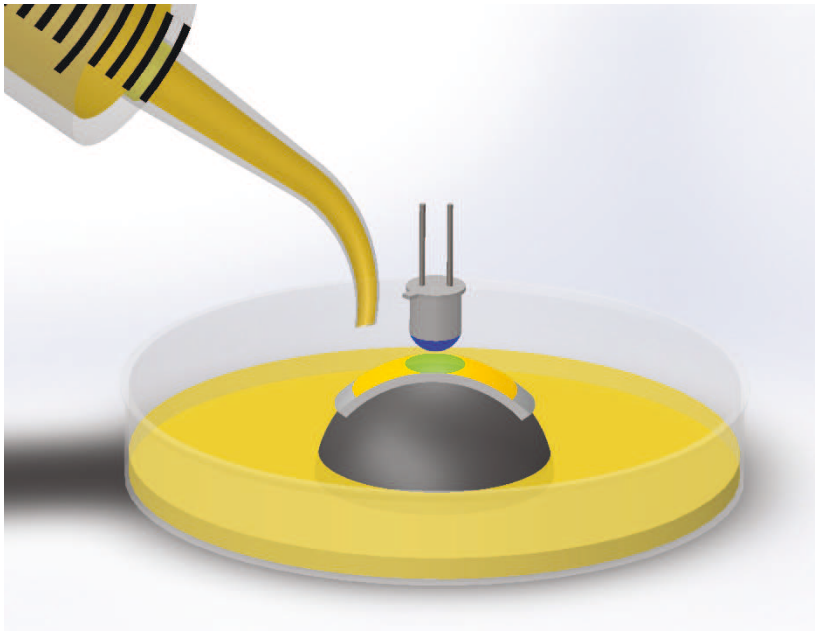
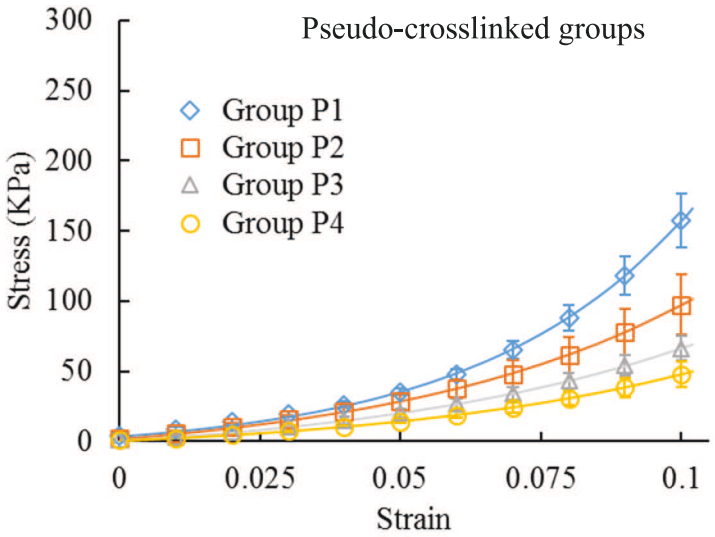


Figure 2

a)


Pseudo-crosslinked groups
Group P1
Group P2
Group P3
Group P4
Stress (KPa)
300
250
200
150
100
50
0
0
0.025
0.05
0.075
0.1
Strain

b)

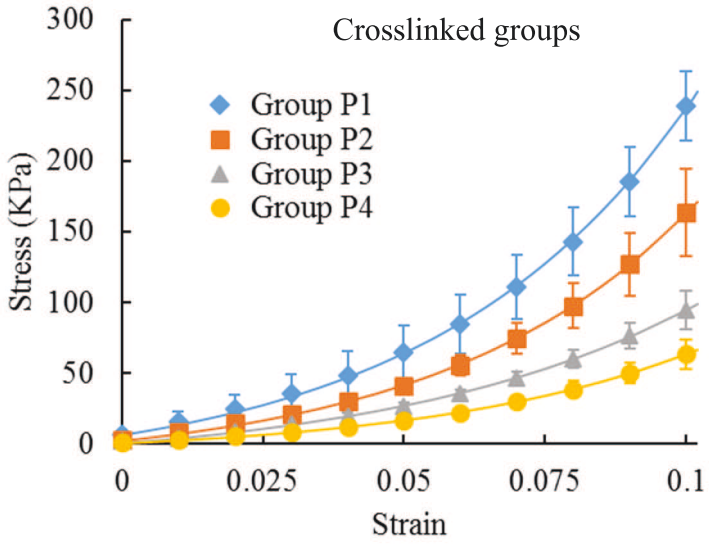
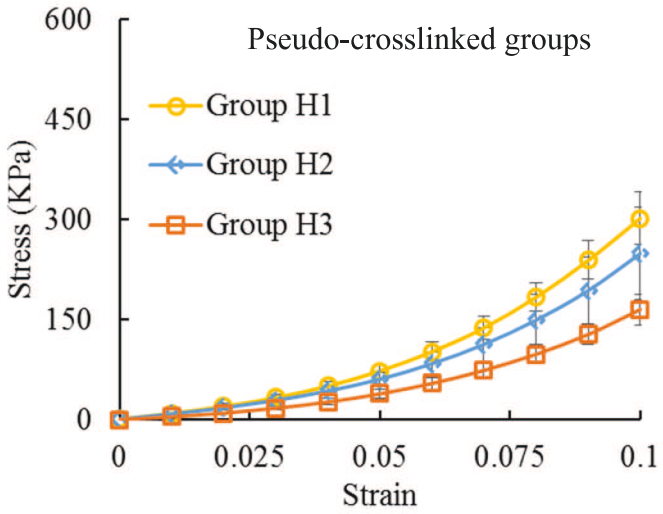

Crosslinked groups
Group P1
Group P2
Group P3
Group P4
Stress (KPa)
300
250
200
150
100
50
0
0
0.025
0.05
0.075
0.1
Strain

Figure 3

b)


Pseudo-crosslinked groups
Group H1
Group H2
Group H3
Stress (KPa)
600
450
300
150
0
0
0.025
0.05
0.075
0.1
Strain

a)

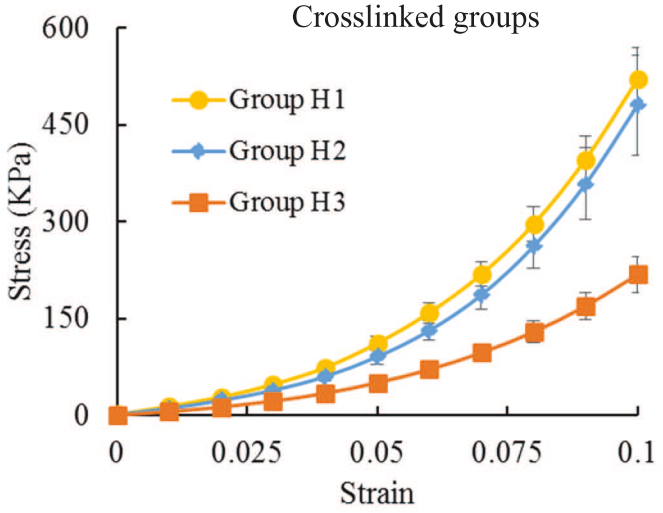

Crosslinked groups
Group H1
Group H2
Group H3
Stress (KPa)
600
450
300
150
0
0
0.025
0.05
0.075
0.1
Strain

Figure 4

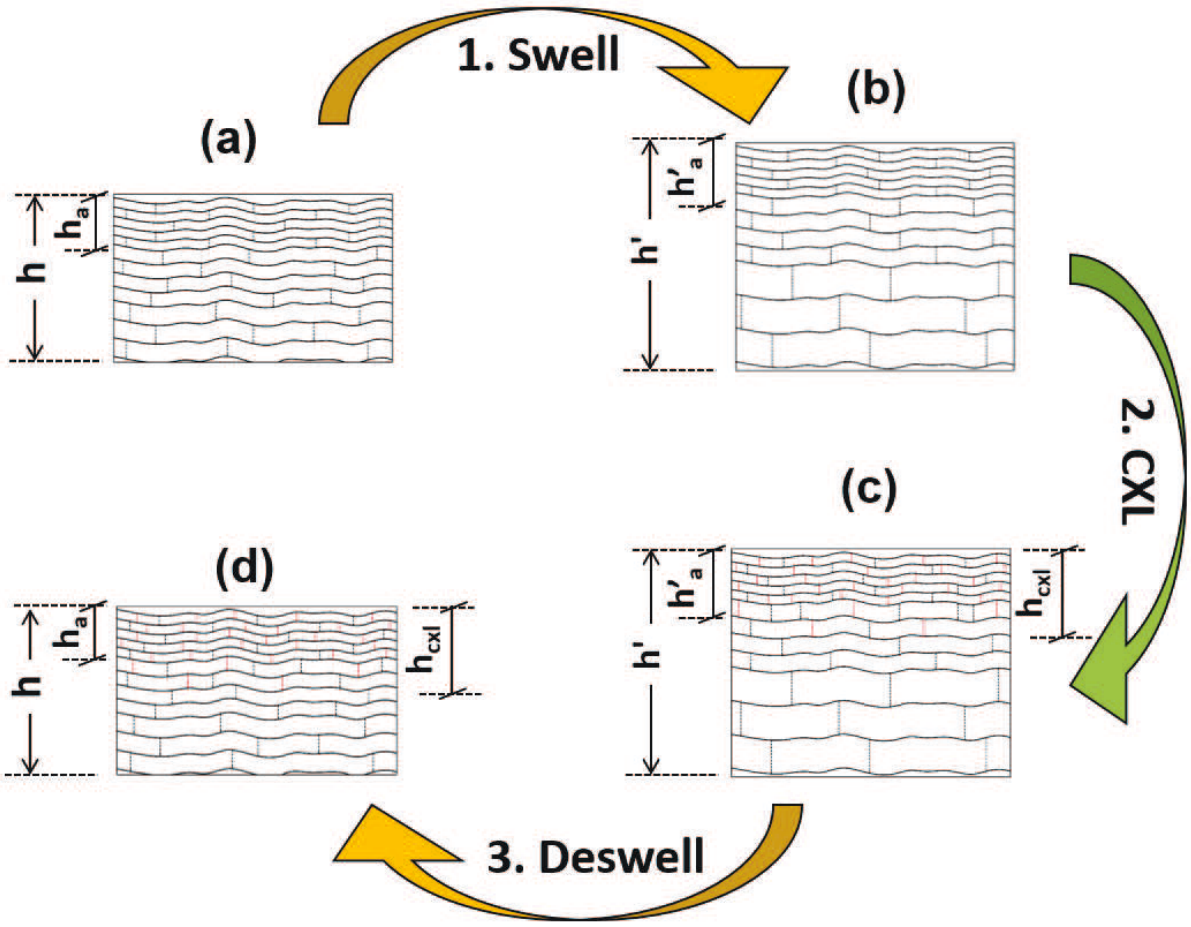
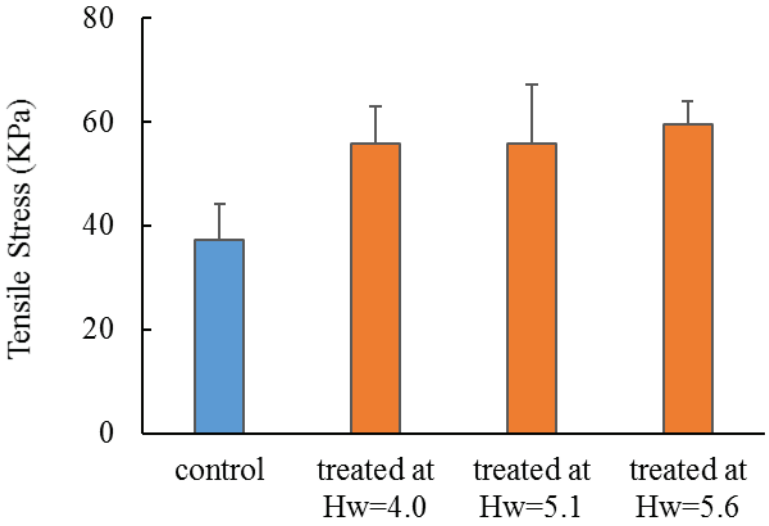


Figure 5

a)


Tensile Stress (KPa)
80
60
40
20
0
control
treated at Hw=4.0
treated at Hw=5.1
treated at Hw=5.6

b)

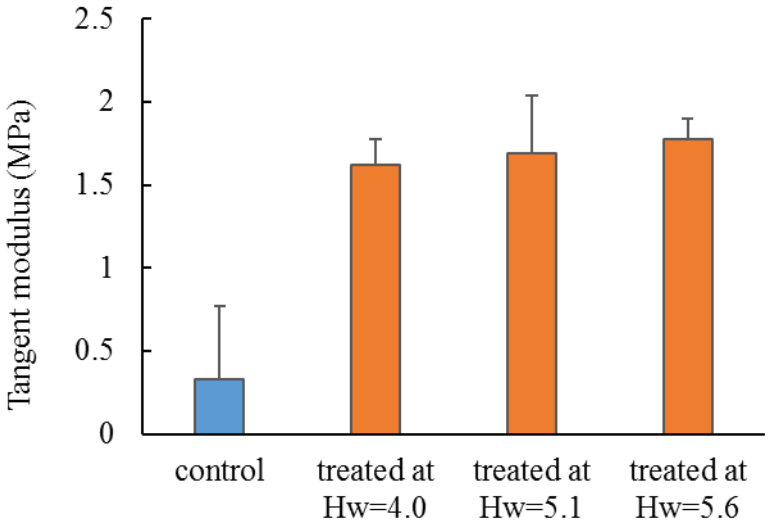
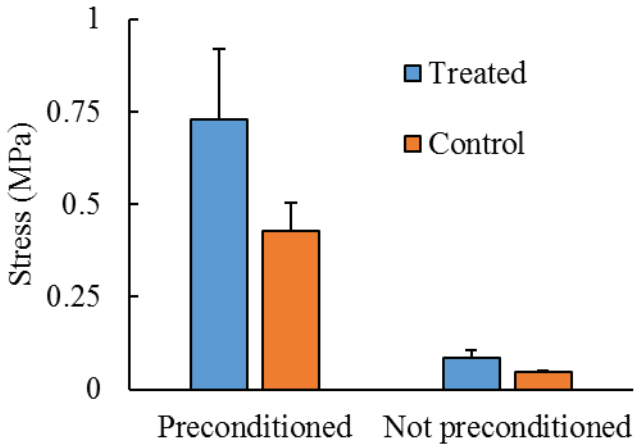

Tangent modulus (MPa)
2.5
2
1.5
1
0.5
0
control
treated at Hw=4.0
treated at Hw=5.1
treated at Hw=5.6

Figure 6

a)


1
0.75
0.5
0.25
0
Stress (MPa)
Treated
Control
Preconditioned
Not preconditioned

b)

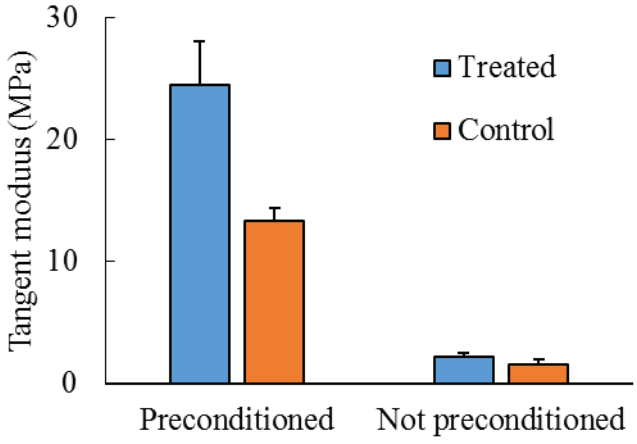
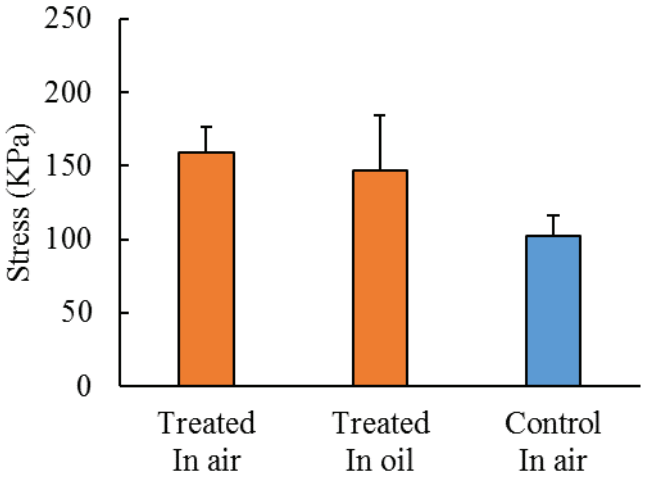
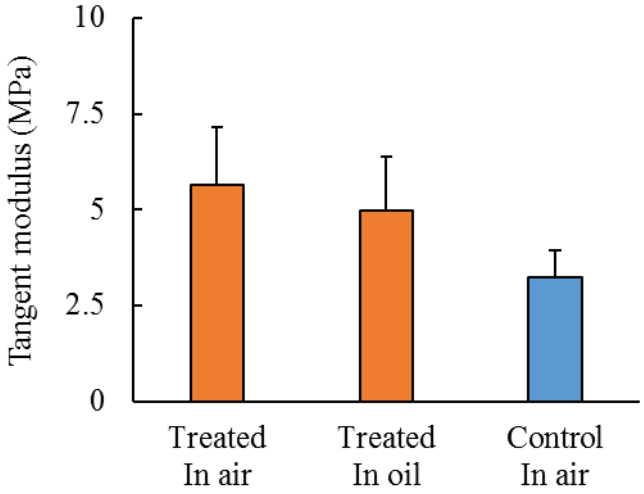

30
20
10
0
Tangent moduus (MPa)
Treated
Control
Preconditioned
Not preconditioned

Figure 7

a)


250
200
150
100
50
0
Stress (KPa)
Treated
In air
Treated
In oil
Control
In air

b)


10
7.5
5
2.5
0
Tangent modulus (MPa)
Treated
In air
Treated
In oil
Control
In air

Table

Groups			Dextran Concentration	Thickness (μm)	Hydration	ε=6%	ε =8%	ε =10%
Porcine samples	P1	treated	20%	600 ± 40	3.3 ± 0.2	85 ± 21	143 ± 24	239 ± 25
		control				48 ± 4	88 ± 9	158 ±19
	P2	treated	10%	760 ± 25	3.3 ± 0.2	56 ± 7	98 ± 16	163 ± 31
		control				37 ± 7	62 ± 13	97 ± 21
	P3	treated	5%	1085 ± 25	5.1 ± 0.1	36 ± 3	60 ±6	94 ± 13
		control				26 ± 4	43 ± 6	66 ± 9
	P4	treated	2.5%	1300 ± 50	5.6 ± 0.1	22 ± 3	39 ± 6	63 ± 10
		control				19 ± 2	31 ± 5	48 ± 9
Human samples	H1	treated	20%	550 ± 15	3.2 ± 0.1	159 ± 16	296 ± 27	521 ± 48
		control				101 ± 14	184 ± 22	302 ± 40
	H2	treated	15%	650 ± 30	3.9 ± 0.2	132 ± 15	262 ± 34	481 ± 77
		control				85 ± 16	150 ± 38	249 ± 70
	H3	treated	10%	850 ± 45	5.3 ± 0.3	71 ± 8	129 ± 17	218 ± 28
		control				55 ± 9	98 ± 13	166 ± 23

Table 1. Dextran concentration, thickness, and hydration, and tensile stress in KPa at 6%, 8%, and 10% strain for control and treated porcine and human corneal strips.

Groups			ε=6%	ε =8%	ε =10%	A(KPa)	B
Porcine samples	P1	treated	2.35 ± 0.32	4.04 ± 0.39	5.89 ± 0.51	28 ± 8	22.3 ± 2.0
		control	1.36 ± 0.22	2.43 ± 0.42	4.03 ± 0.62	15 ± 1	24.2 ± 1.6
	P2	treated	1.56 ± 0.34	2.57 ± 0.68	4.29 ± 0.81	19 ± 1	22.4 ± 1.6
		control	1.21 ± 0.39	1.64 ± 0.42	2.33 ± 0.64	10 ± 0	22.7 ± 1.2
	P3	treated	0.98 ± 0.17	1.45 ± 0.34	2.12 ± 0.43	12 ± 1	21.8 ± 2.0
		control	0.68 ± 0.15	1.02 ± 0.17	1.34 ± 0.24	7 ± 1	23.0 ± 1.4
	P4	treated	0.65 ± 0.08	1.03 ± 0.26	1.41 ± 0.40	9 ± 3	20.5 ± 2.9
		control	0.44 ± 0.15	0.73 ± 0.27	0.98 ± 0.32	6 ± 1	21.5 ± 1.9
Human samples	H1	treated	5.64 ± 1.50	11.39 ± 2.40	15.86 ± 2.75	42 ± 3	26.0 ± 1.4
		control	3.24 ± 0.71	5.21 ± 1.36	8.03 ± 2.80	27 ± 5	24.7 ± 1.6
	H2	treated	4.54 ± 0.84	8.89 ± 2.19	13.45 ± 2.36	38 ± 7	25.8 ± 1.5
		control	2.57 ± 0.91	3.94 ± 1.21	6.19 ± 2.23	25 ± 5	23.5 ± 1.3
	H3	treated	2.41 ± 0.51	3.30 ± 0.56	5.58 ± 0.92	20 ± 1	24.3 ± 0.8
		control	1.64 ± 0.29	2.50 ± 0.23	4.61 ± 2.11	18 ± 6	23.4 ± 3.6

Table 2. Tangent modulus in MPa at 6%, 8%, and 10% strain, and the fit parameters for control and treated porcine and human corneal strips. The coefficients of determination were larger than 99% for all groups. The hydration and thickness of different groups are given in Table 2.