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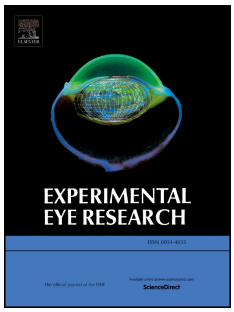

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Depth-dependent mechanical properties of the human cornea by uniaxial extension

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ABSTRACT

The purpose of this study was to investigate the depth-dependent biomechanical properties of the human corneal stroma under uniaxial tensile loading. Human stroma samples were obtained after the removal of Descemet's membrane in the course of Descemet's membrane endothelial keratoplasty (DMEK) transplantation. Uniaxial tensile tests were performed at three different depths: anterior, central, and posterior on 2 x 6 x 0.15 mm strips taken from the central DMEK graft. The measured force-displacement data were used to calculate stress-strain curves and to derive the tangent modulus. The study showed that mechanical strength decreased significantly with depth. The anterior cornea appeared to be the stiffest, with a stiffness approximately 18% higher than that of the central cornea and approximately 38% higher than that of the posterior layer. Larger variations in mechanical response were observed in the posterior group, probably due to the higher degree of alignment of the collagen fibers in the posterior sections of the cornea. This study contributes to a better understanding of the biomechanical tensile properties of the cornea, which has important implications for the development of new treatment strategies for corneal diseases. Accurate quantification of tensile strength as a function of depth is critical information that is lacking in human corneal biomechanics to develop numerical models and new treatment methods.

Keywords: Human cornea, tensile testing, depth-dependent, uniaxial testing.

1. INTRODUCTION

Over 185,000 corneal transplant surgeries are performed annually worldwide, yet, 53% of the world's population does not have access to donor corneas, which also limits their availability for research (Bunya et al., 2023). Understanding the biomechanical properties of the cornea can help us develop more effective surgical interventions and treatment strategies that can restore corneal integrity and prevent the progression of corneal diseases. It is critical to understand the biomechanics of this complex tissue, which is highly anisotropic, heterogeneous, and multi-layered. Knowledge of the tensile strength of the cornea can help improve treatment strategies for conditions that affect the structural integrity of the cornea, such as keratoconus, corneal ectasia, and corneal ulcers. For example, measuring corneal resistance to deformation or stress can help us determine the optimal parameters for corneal crosslinking to strengthen corneal tissue in keratoconus, or design keratotomies to appropriately reduce corneal astigmatism. In addition, studying the tensile strength of corneal tissue can also contribute to the development of new biomaterials, implants and surgical techniques for corneal reconstruction and transplantation (Roberts, 2016).

While full-thickness tensile testing has been performed on human corneal strips (Elsheikh et al., 2008; Hoeltzel et al., 1992; Mahdian et al., 2021), there is limited information in the literature on the depth-dependent biomechanics of the human cornea, particularly on tensile properties. Existing studies on depth dependence have been performed using shear (Petsche et al., 2012; Sloan et al., 2014), compression (Ramirez-Garcia et al., 2018), cohesive tension (Randleman et al., 2008), and indentation (Labate et al., 2015) methods. Physiologically, however, the cornea is subjected to tension due to the intraocular pressure, which is most important for understanding its biomechanics.

Therefore, this study aims to characterize the mechanical properties of the human corneal stroma at different depths, corresponding to the anterior, central, and posterior corneal stroma by uniaxial extension. To our knowledge, this is the first depth-dependent uniaxial tensile study performed on

human corneas. To control sample preparation, strips were cut with a femtosecond laser to ensure uniform geometry and thickness and to precisely control the depth of sample extraction.

MATERIALS AND METHODS

2.1 Experimental setup

Nine human donor corneas (age 55-79 years) were examined in this study. Since good quality corneal tissue is scarce, this study used corneas after the removal of Descemet's membrane (DM) for DMEK surgery. The corneas including a 3 mm scleral ring, were provided by the Department of Ophthalmology of the Inselspital Bern immediately after peeling the DM, which was performed without damaging the posterior corneal stroma. Before DM peeling, the corneas were examined for corneal scars and de-swollen in Carry-C (Alchimia, Italy) for 24 hours. After DM peeling, the corneas were placed in a hydration preserving culture medium containing 5 % Dextran solution. Since this study used tissue from decreased donors that would otherwise be discarded, the IRB (Cantonal Ethics Committee of the Canton of Bern) granted exempt status. The orientation of the corneas were unknown.

Within 72 hours after DM peeling, the cornea was placed in an artificial anterior chamber pressurization device with the corneal epithelium side facing upward to prevent movement during strip cutting with the Femto LDV Z8 Neo femtosecond laser (Ziemer Ophthalmic Systems AG, Switzerland) (Figure 1). The laser handpiece was centered on the cornea using the OCT integrated into the device and moved only after all strips were cut. The OCT was also used to measure the total corneal thickness. The average central corneal thickness in this study was 770 µm. The high thickness variation can be attributed to swelling, despite the deswelling media due to lack of the endothelium layer. For each eye, three strips 6 mm long, 2 mm wide, and 0.15 mm thick were cut in the central-corneal region from the anterior (D1), central (D2), and posterior (D3) thirds of the cornea. No epithelium was present on the prepared samples. In all but two samples, the first 100 µm of cornea were discarded to ensure that the Bowmann's membrane was not included in the test. In the two excluded samples, it was not possible to omit this layer because of low corneal thickness, so the anterior sample (D1) was taken just below the epithelium. However, no differences were observed in the tests. The samples were

individually preserved and tested in a hydration-preserving culture medium containing 5 % Dextran to maintain the sample dimensions after cutting (Hamon et al., 2021; Wolf et al., 2009).

The strips were uniaxially tested in a bath containing the same culture medium using the Ustretch device (CellScale, Waterloo, Canada) at room temperature (Figure 2). The tissue was pre-stretched with a low force of 10 mN to ensure uniform tension across the samples. The samples were then loaded with 6 cycles at a strain rate of 0.16 % /s. The fifth cycle of force-displacement data was recorded for analysis as the mechanical response of the tissue stabilized after the fifth preconditioning cycle. The specimens were tested up to a strain of 10 %, which is consistent with previous experiments (Boschetti et al., 2012; Elsheikh et al., 2011, 2008; Wollensak et al., 2003). The actual length of the specimen was used to calculate the strain, taking into account the distance between the grips under the pre-stretch 10 mN, which differs from the original distance of 5 mm due to the initial tensioning of the testing device and the unfolding of the specimen. For this reason, the reference length of the sample was calculated as the actual distance of the grip at the start of the 5th cycle. The strain was then calculated as the ratio of extension to this reference length of the sample. The stress was determined by dividing the force by the original cross-sectional area, specifically the product of the thickness of the sample (0.15 mm) and the width at the attachment of the rakes (1.56 mm).

The relationship between stress and strain was further characterized using an exponential function based on a previously proposed method for studying corneal inflation (Eliasy et al., 2019; Elsheikh et al., 2010):

$$\sigma = A\left(e^{B(\varepsilon-\varepsilon_0)} - 1\right) \quad (1)$$

Here, A and B are material parameters, and ε_0 is determined such that the stress corresponds to our experimental measurement at zero strain. This additional strain is necessary to account for the initial experimental pre-stress introduced to remove the initial compliance of the measurement setup. Finally, the stiffness of the specimen was calculated as the derivative of the fitted exponential function and used to quantify the stiffness ratio between the different depths.

120 **2.2 Statistical Methods**

121 Statistical analysis was performed using Python 3.7 (Scipy.stats). The uniaxial force and extension data
122 were used to generate stress-strain curves for each depth and sample. Tangent moduli were
123 calculated for the low (2 %), intermediate (5 %), and high-strain (10 %) regions of the curves,
124 corresponding to the stiffness of the tissue at different depths. A one-way ANOVA was performed on
125 the tangent moduli data for each depth group to analyze the difference between depths. Due to
126 sample exclusion during experimental measurements, our sample size was insufficient to perform
127 paired statistical tests. A Tukey post hoc test was used to determine which groups were significantly
128 different. A p-value of < 0.05 was considered significant.

2. RESULTS

Twenty-seven strips were cut from the 9 donor corneas, but 4 strips were excluded from analysis, bringing the total number of samples for all groups to 23. The main reason for exclusion was damage to the sample – usually in the area of attachment – or loosening at the attachment points. 7 samples were available in the D1 group, and 8 in each of the D2 and D3 groups.

The force-displacement behavior during uniaxial measurement was determined for the three depths. The corresponding stress-strain data was calculated from the measurements (Figure 3). Besides the typical viscous and nonlinear relationship, our results showed that the mechanical properties of the stroma decreased with depth, with the anterior D1 group having the highest force, then the central D2 layer, and the lowest in the posterior D3 stromal layer. The highest variability in stress was observed in the D3 group (Figure 3). Statistically, D1 and D2 showed a significant difference in force ($p < 0.001$), as did D2 and D3 ($p < 0.001$) and D1 and D3 ($p < 0.001$). An average maximum force of 0.22 N was observed in the D1 group, 0.18 N in the D2 group and 0.14 N in the D3 group at 10 % strain. This corresponds to a decrease of 18 % mechanical strength in the central layer (D2) and a 36 % in the posterior layer (D3), as compared to the anterior layer (D1).

The stress- strain data were used to calculate the tangent modulus at 2 %, 5 % and 10 % strain. The average stress at 10 % strain was used to calculate the difference in mechanical strength between the three layers. A linear decrease in mechanical properties was observed across depth. The tangent moduli were evaluated at different strain levels (Figure 4). At 5 % strain, groups D2 and D3 showed no significant difference ($p = 0.051$), but at 10 % this changed to a significant difference ($p < 0.001$). At all strains, the tangent modulus was significantly higher at D1 than D2 and D3 ($p < 0.001$). We found no correlation of tangent modulus with age or thickness.

Exponential fitting of stress-strain data showed good agreement with experimental data for all the depths (Figure 3). Interestingly, the value of exponent B remains nearly constant across different depths, implying that the mechanical properties between depths are primarily determined by the

linear parameter A over a strain range from 0% to 10% (Table 1). The relative change in corneal stiffness between depths was quantified by calculating the normalized stiffness with respect to the anterior depth. Since the exponential parameter B remains constant at all depths, this relationship is nearly constant at each strain level. Furthermore, stiffness decreases linearly from the anterior to the posterior. The central cornea has a stiffness of about 80% of the anterior stiffness, while the posterior layer is 60% (Figure 5).

3. DISCUSSION

The objective of this study is to characterize the mechanical properties of the human corneal stroma at different depths, corresponding to the anterior, central, and posterior cornea by uniaxial extension. The study aims to understand the depth-dependent biomechanical behavior of the cornea under tensile loading, which is one of the most relevant loading situation for physiological conditions (Kling et al., 2020). While the physiological intraocular pressure is better represented by a biaxial setting, uniaxial testing is a widely used method for estimating tensile mechanics. This study is the first to characterize the depth-dependent biomechanics of the human cornea under tensile loading. It should contribute to a better understanding of the biomechanical properties of the cornea and allow the development of new treatment strategies for corneal diseases.

In this study, a linear decrease in tensile mechanical strength was observed across the depth of the cornea. The central layer was found to be 18 % weaker than the anterior cornea and the posterior cornea was found to be 38 % weaker than the anterior cornea.

Other depth-dependent mechanical characterizations found in the literature include shear (Petsche et al., 2012; Sloan et al., 2014), compression (Ramirez-Garcia et al., 2018), cohesive tensile (Randleman et al., 2008), and indentation (Labate et al., 2015). Direct comparison of measured properties is not possible because the metric used to characterize the mechanical response is different for each test method. Qualitatively, all these studies found significant stiffer properties for anterior cornea than the posterior cornea. However, these studies report that the central stroma is mechanically similar to the posterior cornea, which is different from our results, where significant differences in the mechanical behavior were observed at all measurement depth – anterior, central, and posterior – with the stiffness linearly decreasing across depth. This difference in analysis may be attributed to the type of loading applied to samples in the different tests. The tensile strength of the collagen bundles is the primary contributor to the uniaxial forces, which is orders of magnitude higher in tension than in compression. As such, compression tests predominantly characterize the response of the stromal

matrix rather than the properties of the collagen fiber, which can explain these differences (Sun, 2021).

In this study, the posterior cornea was found to be 38% less stiff than the anterior cornea, which is in a similar range to the depth dependent tensile mechanics of the porcine cornea (Nambiar et al., 2022). The central layer was found to be 18% less stiff than the anterior cornea, which was different from the trends in the porcine cornea that showed a similar mechanical strength of the central and anterior layers (Nambiar et al., 2022). Comparing the results of this study with uniaxial measurements on full thickness human corneal strips show that stresses of full thickness measurements are in the range of D2-D3 groups of this study (Elsheikh, A. et al., 2008; Hoeltzel et al., 1992; Mahdian et al., 2021). The deviations can be attributed to the depth dependence of the material and to differences in the test setup, such as clamping and preconditioning. The parameters obtained by fitting the exponential function indicate a more linear response of the tissue compared with the values published for inflation experiments (Eliasy et al., 2019; Elsheikh et al., 2010). This difference could be due to the different loading methods used in the two studies, especially since the inflation experiments reflect a predominantly biaxial loading scenario. This comparison indicates that uniaxial tests should be combined with biaxial loading to obtain a comprehensive description of the corneal mechanical response.

Accurate quantification of the tensile strength in a depth-controlled manner is critical information that is lacking in current numerical models developed to study various refractive interventions (Ariza-Gracia et al., 2017; Pandolfi and Manganiello, 2006; Sinha Roy et al., 2014; Studer et al., 2010). Even if the mechanical properties identified in this study corresponds to aged corneas, the trends of reduction in strength quantified in this study can be reproduced for applications in the young cornea, which is supported by imaging data and the other mechanical tests in literature (Labate et al., 2015; Petsche et al., 2012; Ramirez-Garcia et al., 2018; Randleman et al., 2008; Sloan et al., 2014).

Looking at the ultrastructure of the human cornea, we see that collagen fibers of the anterior stroma are randomly dispersed in the plane of the cornea and aligned along the curvature (Quantock et al., 2007). This in-plane dispersion becomes preferentially oriented in the nasal-temporal and superior-inferior directions with increasing depth, while preserving some level of isotropic scatter (Abass et al., 2015; Quantock et al., 2007). However, the posterior cornea exhibits lower mechanical properties, which seems contradictory because the fibers carry most of the load. This behavior could indicate lower fiber density in the posterior cornea. The lower stiffness could also be due to a greater out-of-plane dispersion of the fibers in the posterior cornea, resulting in a lower contribution of the fibers to the tensile stress. It is plausible that a combination of these elements are at play.

This also makes the lack of orientation markers on the donor tissue a limitation of this study for the posterior group (D3), and a possible explanation for the larger variability in the properties measured in this group. Another reason for the larger variability observed in the mechanical measurements in the posterior group could be related to fact the posterior stroma is more susceptible to swelling (Meek et al., 2003; Wang et al., 2004), especially after removal of the DM. Although care was taken to maintain the level of hydration of the specimens during testing, the corneas remained slightly swollen when the specimens were cut from the cornea with the laser. This problem is common in ex vivo mechanical testing. Although this may result in a slight underestimation of the measured stresses, the trends and magnitude of the decrease in mechanical strength with depth remain valid. Another simplification in this study is the assumption that the strips are flat, although the cornea has a curvature. However, we suspect that the effect on the measured properties is small because of the small dimensions of the specimens-particularly their thickness-and the fact that they flatten under their own weight when attached to the experimental setup. Since the corneas were obtained from transplant tissue, they were from elderly donors, which limits the measured properties to the elderly population and prevents conclusions about age-dependent mechanical behavior. We found no correlation of tangent modulus with age, which may be attributed to the limited age variation within the measured samples.

This study contributes to the growing body of knowledge on the biomechanics of the human cornea by quantifying the depth-dependent tensile biomechanics. This work also highlights the importance of considering depth-dependent mechanical properties when planning and evaluating corneal treatments. The results from this study could inform the development of better numerical models for refractive interventions and improve our understanding of corneal diseases. The method by which corneal stroma was derived in this study from DMEK surgical waste can be an important source of corneal stroma for future research.

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328

Depth	A (MPa)	B (-)	ε_0
D1	64.20 10^{-3}	25.28	11.19 10^{-3}
D2	47.41 10^{-3}	25.54	14.35 10^{-3}
D3	25.64 10^{-3}	26.74	22.37 10^{-3}

Table 1: The estimated parameters from the exponential fitting of the experimental stress strain data showed a linear decrease in parameter 'A' while parameter 'B' remained relatively constant. Parameter ε_0 was obtained to account for the prestress in the experimental condition at 0 strain.

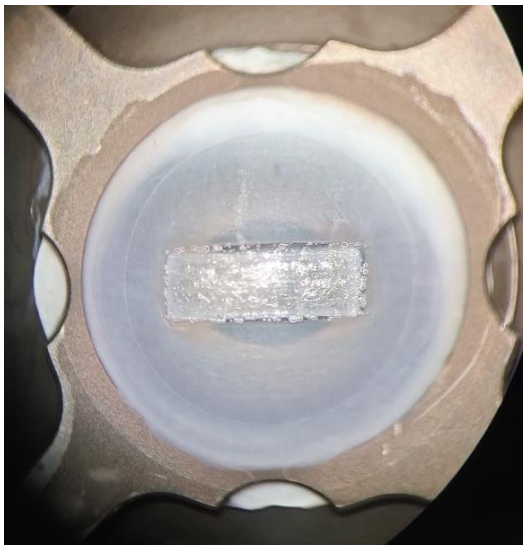

Figure 1: The cornea with scleral rim, clamped in an artificial chamber showing the strips cut out in the mid corneal region.

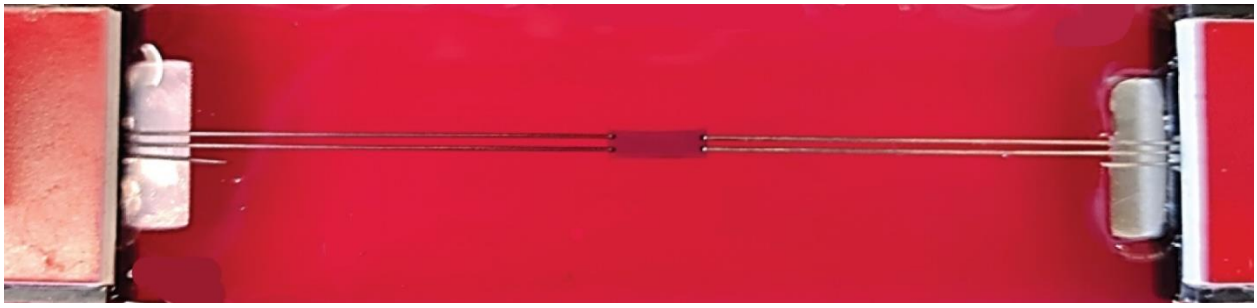

Figure 2: The corneal strip (6 x 2 x 0.15 mm) was tested under traction in a bath of MEM + 5% dextran with BioRake attachments. Note that one of the tins has been cut on both sides to leave only two for sample attachment.

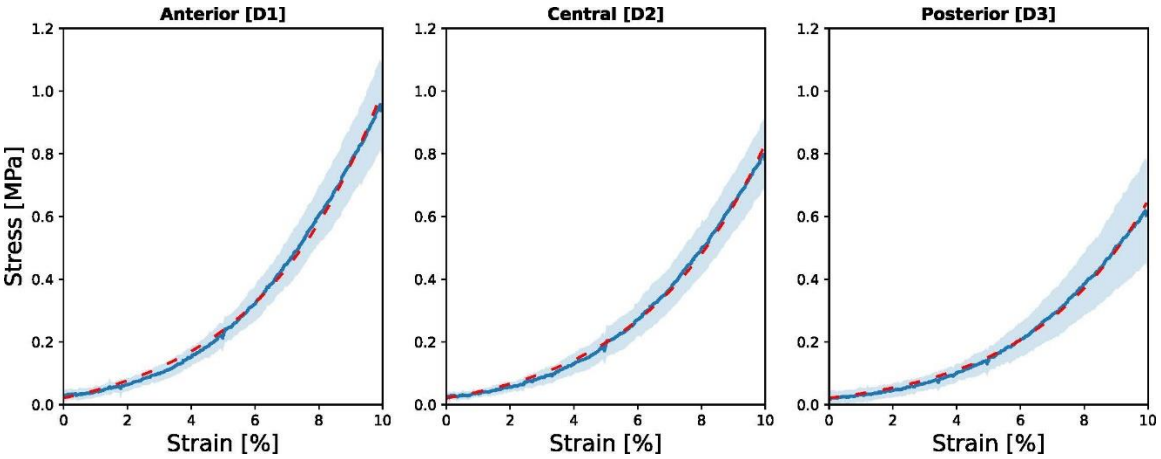

Figure 3: The Stress-Strain reaction of the strips in the anterior (D1, n=7), central (D2, n=8) and posterior (D3, n=8) groups. The solid blue line represents the mean stress, and the shaded region represents the standard deviation. The dashed red line represents the exponential fit with equation 1.

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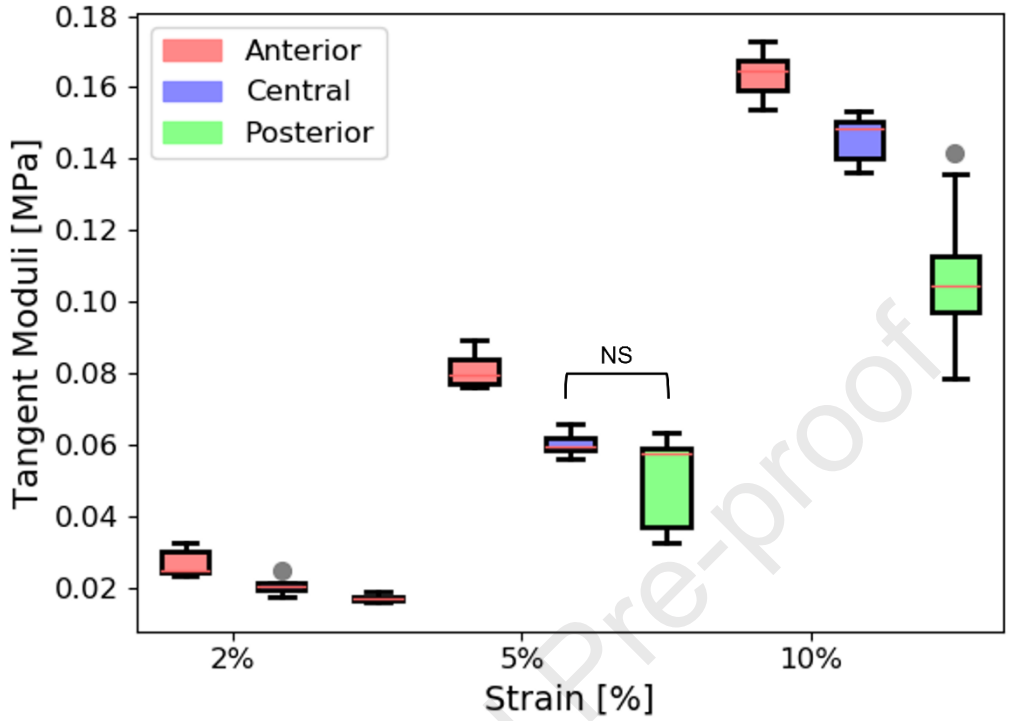

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351 **Figure 4:** The tangent moduli in the anterior (D1, n=7), central (D2, n=8) and posterior (D3, n=8)
352 groups, for 2%, 5% and 10 % strain. All groups except indicated (NS, $p = 0.051$) show a significant
353 difference ($p < 0.05$).

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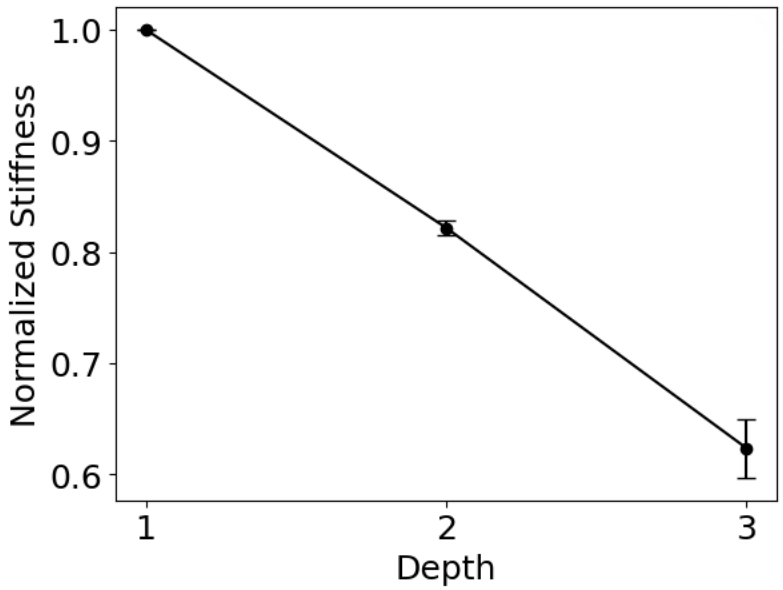

Figure 5: The stiffness calculated based on the exponential fit at each depth was normalized with respect to the stiffness of the anterior layer. This normalized stiffness ratio showed a linearly decreasing stiffness from the anterior to the posterior part of the cornea.

Highlights

- Human cornea shows depth-dependent mechanical properties, with a linear decrease in tensile mechanical properties from the anterior to the posterior cornea.
- The tangent moduli at different strains show significant differences across the depth of the cornea.
- While full-thickness studies have been previously performed, this to the best of our knowledge, is the first depth-dependent analysis in uniaxial extension of the human cornea