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Biomechanical behaviour – Anisotropy of eye cornea through experimental strip tests

Biomechanical behaviour – Anisotropy of eye cornea through experimental strip tests

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Abstract. With the advent of research it was identified that material properties are responsible for errors in tonometry pressure (referred to as Goldmann IOP or IOPG) with the stiffening of a composite structure of corneal tissue in particular. Strip tensile tests are conducted to determine their stress–strain relationship for the purpose to study the behaviour of material properties of cornea. Specimens are taken from the superior–inferior (vertical) and temporal–nasal (horizontal) directions. Testing is performed on an Instron machine, under different rate of loading conditions. First set of experiment, with single strain rate, is executed on eyes having random population. While the second set of experiment is executed on eyes of the same animal in both directions, and different strain rates are applied each specimen. Relatively, the first set of experiment is found to be slightly different and less accurate. In general, it is found that the vertical specimen is 34% on an average stiffer than the horizontal specimen compared to Kampmeier et al. of 20% (studied in 2000) and Defu Wang of 15% (studied in 2007). Curve fitting coefficients are also evaluated for 4–degree polynomial. The anisotropy is evident by plotting the ratio of E–tangent value of vertical Ev and horizontal Eh against stresses with individual strain rates. The value of Ev/Eh increases with slightly slow rate with stresses as compared to achieved through slow strain rates.

1. Introduction

Many researchers apply structural engineering analysis tools for the development of numerical models, while others direct their efforts on the study of behaviour of material properties such as viscoelasticity, hyperelasticity and anisotropy [1, 2, 3]. The field of ophthalmology is one area of medical science that is currently benefiting from these applications. To demonstrate mechanics of cornea, previous studies typically employed a tensile strip method [4, 5, 6, 7], or an inflation method [8, 9]. With the advent of research it is identified that material properties are responsible for errors in IOPG [10, 11] with the stiffening of corneal tissue in particular [1, 2]. In relation to the study on numerical model by Khan [12], the stress (σ)–strain (ε) relationships holding within the material model, gathered through the testing of excised cornea (tested for age range of 50–95 years old). However, the strip tensile tests are different from inflation tests (useful for Glaucoma), and can be used to identify more focused observations specifically useful to provide treatment of eye disease and

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injury, and for predicting the effects of clinical surgery, develop numerical simulation of refractive surgery. Hence, it becomes vital to conduct tests on fresh samples and at various loading rates.
The arrangement of collagen fibril in the corneal stroma can play critical role in strip tensile testing; attempts have been made to identify the geometries [13, 14, 15].
With increasing age there is an increase in the cross-sectional area of fibrils due to the continual deposition of collagen [14] and an increase in glycation-induced cross-linking between fibrils. The nonlinear behaviour of the cornea has been confirmed by strip testing results as early as 1968 when Nyquist [6] investigated the biomechanical behaviour of porcine corneas.
Daxer et al. [14] & Boote et al. [16] estimated that two-thirds of the stromal fibrils are aligned within the 45° sectors surrounding the two main orthogonal directions and the remaining third are oriented within the oblique sectors in between them. Wang [3] identified two preferential directions within which most fibrils run, namely the superior-inferior and the temporal-nasal directions; therefore, test-strips are extracted for these locations.

2. Method and materials

2.1. Method
Several of the studies [5, 6, 17, 18] used porcine corneas although others [4, 19], less commonly, adopted rabbit and bovine corneas. Zeng et al. [7] established that porcine corneas are physiologically close to human corneas. Hence, for present study, two sets of specimens are taken from the superior-inferior (vertical) and temporal-nasal (horizontal) directions of porcine corneas for testing conducted at room temperature on an Instron 3366 materials testing machine (load cell capacity = 50 N). Behaviour is studied under different rate of loading conditions, storing readings at every 0.1 s.
Due to oval shape of porcine cornea, the experimental clamping setup is such that for the horizontal strip, the clamps are extended to grip corneal-tissue ends, while the clamps are to be terminated at sclera-tissue to maintain a clear specimen strip length of 12 mm.
Only single strain rate is applied on first set of specimens (refer Table 1) obtained from random population of eyes.

Table 1. First set of specimens.

Specimen nos.	Axis of testing	Rate of Loading	No. of conditioning cycles	Max. load during conditioning
		mm/min		N
42, 45, 49, 62, 98, 102	V	0.1	3	1.4
46, 103, 110, 111, 112, 113	H	0.1	3	1.4
48, 50, 51, 52, 53, 54	V	1	3	1.4
104, 105, 106, 107, 108, 109	H	1	3	1.4
58, 59, 60, 61, 63, 64	V	3	3	1.4
83, 84, 85, 86, 87, 88	H	3	3	1.4
43, 44, 47, 55, 56, 57	V	5	3	1.4
77, 78, 79, 80, 81, 82	H	5	3	1.4
65, 66, 67, 68, 69, 70	V	10	3	1.4
89, 90, 91, 99, 100, 101	H	10	3	1.4
71, 72, 73, 74,75, 76	V	50	3	1.4
92, 93, 94, 95, 96, 97	H	50	3	1.4

The second set of experiment (refer Table 2) is conducted on specimens from the same animal, one specimen is taken from vertical direction of one eye and another from horizontal direction of other eye. Different strain rates are applied individually on each specimen as shown in Table 2.

The maximum load applied on first set is 1.4 N for 3 cycles; and 14 N for 4 cycles on second set; after which the specimens are loading until failure. Higher stain rates up to 125 mm/min are also applied; although they have been excluded from study due to inaccuracy in results to yield to any conclusion.

Table 2. Second set of specimens.

Specimen nos.	Axis of testing	Rate of Loading	No. of conditioning cycles	Max. load during conditioning
		mm/min		N
119	H and V	0.1	1	14
		1	4	14
		3	2	14
		5	2	14
		10	2	14
		50	2	18
120, 122 (V)	H and V	0.1	1	14
		1	4	14
		3	2	14
		5	2	14
		10	2	14
121, 122 (H), 123, 124	H and V	0.1	1	14
		1	4	14
		3	2	14
		5	2	14
		10	2	14
		25	2	18
		50	2	18

Corresponding stress-stain plots are drawn from the data of load-elongation collected automatically on the connected computer. The geometrical parameters of the specimen are utilised by assumed that the cornea could be approximated as a homogenous spherical structure of an approximately a constant thickness.

2.2. Significance of E – Tangent

Following the recommendations of Buzard [20] to study the nonlinear behaviour of biomaterials, it limits the effect of initial slack in test specimens captured in stress-strain plots (as shown by [21]), and it is useful to compare hyper-elasticity. This method of E – Tangent is utilised for a selective range of stress values (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 MPa) to study the changing nature of stiffness in second set of specimens applied to the full range of strain rates used from 0.1 to 50 mm/min.

2.3. Materials

Following the directions of Wang [3] and due to ease of availability from local abattoirs, specimens are prepared from a fresh sample through to complete setup. To avoid swelling of tissue, corneal ring is put into saline solution for 2-3 minutes to drain out water. This helped in maintaining standards of testing biological tissues as mentioned by Hoeltzel et al. [4]. In addition, to minimise errors during testing due to viscoelasticity, the specimens underwent conditioning cycles; however, most biologic tissues can be treated as pseudo-elastic.

Starting with measuring the size of cornea by Electronic Digital Caliper DC 150 (150 mm scale with inch/mm selection); a pachymeter (DGH Pachmate 55) is used to measure thickness of central cornea, shown in figure 1. The average central thickness ranged between 0.800 mm and 0.900 mm.

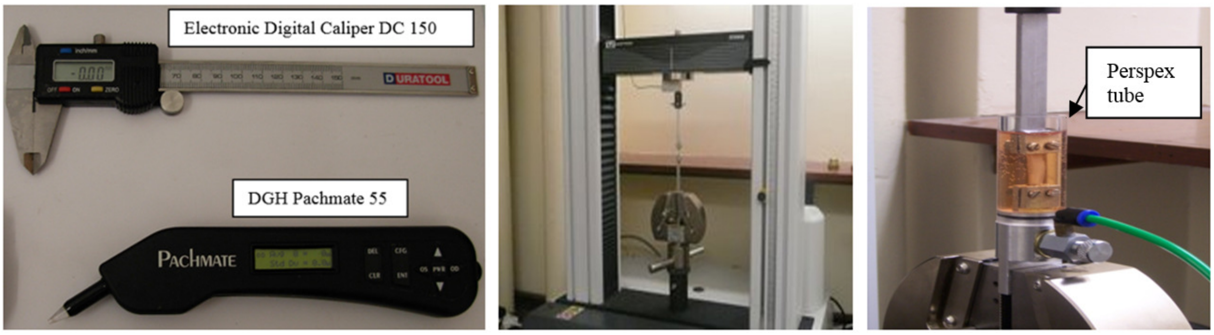

Figure 1. Measuring instrument for cornea and setup.

Slow rates of straining takes approximately two and half hours to failure of specimen; therefore, to avoid swelling and drying of specimen, reusable optinol solution is filled in a leak-proof perspex tube as shown in figure 1 (that is installed around the specimen).

3. Results

Second set of tests (figure 3) are found to be slightly different and more consistent compared with first set (figure 2).

Changing value of E-tangent indicates hyper-elasticity. Although both hyper-elastic in nature, it is clear that the vertical specimen is stiffer than the horizontal specimen at any given instant for high strain rates and after some loading for low strain rates, except for the case with strain rate of 3 mm/min that the behaviour is found to be opposite; this may be due to damaged sample or manual error.

In second set of experiments, the behaviour of cornea is steeper at higher strain rates than at lower strain rates, indicating the presence of viscoelasticity. Generally, the vertical stress-strain behaviour is found to be relatively steeper for both sets.

From figure 3, it is also observed that the specimens attain high failure strength for faster rate of straining than with slower rates.

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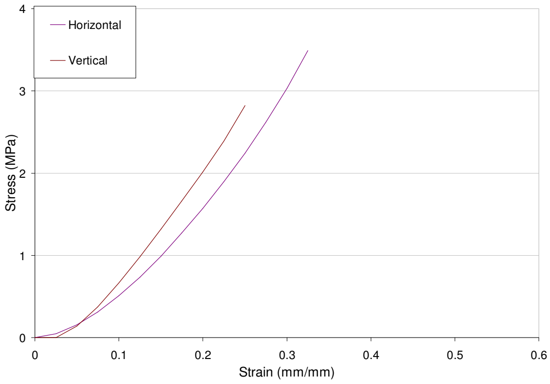
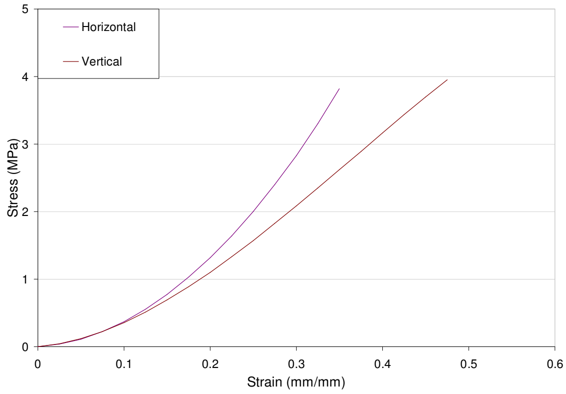
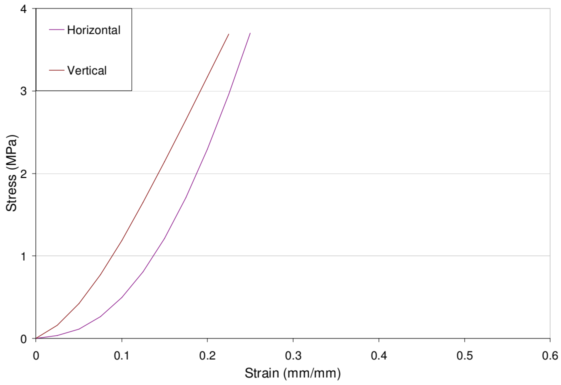


(a) 0.1 mm/min

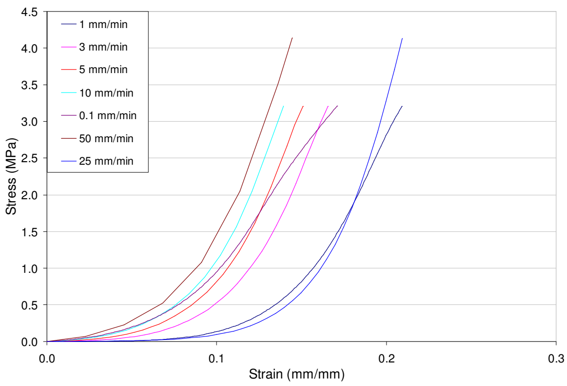


(b) 3 mm/min

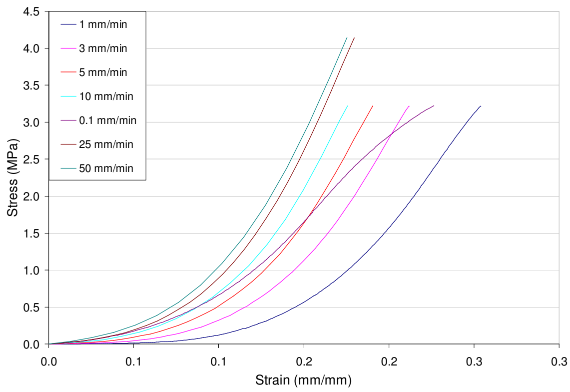


(c) 50 mm/min

Figure 2. Stress strain behaviour of first set with different strain rates in both directions: Vertical (V) verses Horizontal (H).



(a) 121, V



(b) 121, H

Figure 3. Stress strain behaviour of second set with different strain rates in both directions.

3.1. Analysis

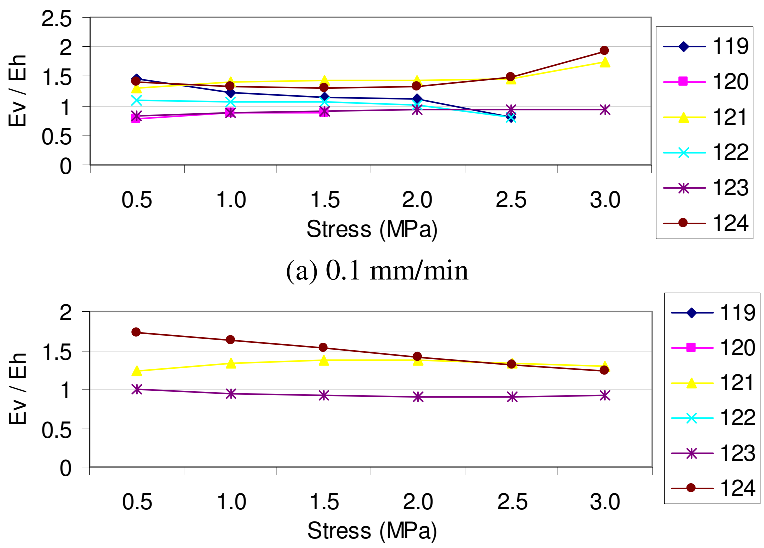
Data analysis is conducted through plotting best fit curve for stress-strain behaviour. For this purpose, polynomial equations having order of magnitude four ($Ax^4+Bx^3+Cx^2+Dx$) are considered. The values of coefficients A, B, C and D generated are assembled in Table 3.

The Ev/Eh verses stress points are plotted in figure 4. Some data is lacking, as is clear from Table 2 that not all pairs underwent full range of loading.

Table 3. Curve fitting coefficients.

Strain rate	Vertical				Horizontal			
(mm/min)	A	B	C	D	A	B	C	D
0.1	1116.7	-678.4	159.4	-3.6	207.9	-150.4	58.3	0.6
1.0	-210.4	84.5	37.5	-0.8	-42.4	-24.5	53.9	-2.0
3.0	1.3	-25.2	26.9	1.1	29.4	-18.9	32.4	0.6
5.0	-173.7	81.1	22.5	3.3	51.0	-117.3	83.1	-3.7
10	2.2	-39.3	42.9	-0.3	71.2	-61	27.7	-0.7
50	298.2	-297.0	108.0	3.7	-372.3	216.1	26.5	0.5

From figure 4, high variations are noticed in Ev/Eh for high strain rates; this is useful to capture rupturing of fibres at smaller scale. It is noticed that this ratio has higher starting value and decreases gradually, indicating visco-elastic response. Whereas for lower strain rates, the trend is increasing after a low initial value. Except for specimen number 123, the ratio Ev/Eh is largely greater than one.



(a) 0.1 mm/min

(b) 50 mm/min

Figure 4. Ratio of Ev and Eh with stresses under different strain rates.

A Ev/Eh ratio higher than one illustrates that the behaviour is stiffer in vertical direction; stressing towards anisotropic response.

4. Conclusion

The comparison between the inferior–superior and nasal–temporal directions improves the understanding of anisotropy present in cornea. In general, with first set, it is found that the vertical specimen is 34% on an average stiffer than the horizontal specimen; in comparison, Wang [3] found that the average stiffness of vertical specimens is 15% more than the horizontal specimen. This variation is may be due to consideration for non-uniform thickness of corneal strip in stress calculations in present study as per [22]. In general, the specimens have shown higher rupture strength in complete failure when tested for vertical direction than for horizontal direction. The changing value of E–tangent in either direction indicates hyper–elasticity. For wide range of strain rates, the pattern of the ratio of E–tangent value of vertical Ev to the horizontal Eh against stresses indicates anisotropy. In general, visco–elastic response is observed for specimens tested at higher strain rates.

In case of vertical specimens, although the cornea is thinnest at its apex, the limbus is seen to rupture to cause failure in all the specimens; whereas, the center of cornea only went negligible decrement. This might also be the reason as to why the vertical specimens are stiffer than the horizontal ones; this is in addition to considering the effect of geometrical variations in calculations. In the field of composite interfaces, this observation highlights the importance of one of the proposition by [23, 24, 25, 26] (although in a different field of composites), that the material response of a composite section are a combined effect of all the materials involved (for example: cornea, limbus, sclera) and largely a function of weakest component after fracture initiation. In the light of this, vertical specimens (clamped on sclera tissue) may show stiffer response as the sclera is relatively stiff; this is not pure corneal tissue behaviour, unlike horizontal specimens. The rupture at limbus may be associated with the geometrical pattern of stromal fibrils, where the longitudinal fibrils largely terminate around the periphery of corneal, similar to as proposed in literature [13] and to the similar findings [21].
The findings of this work can be associated with any surgery on cornea using mechanical instruments with different strain–rate capacities, such that the stresses and strains developed on cornea should be maintained according to its anisotropic behaviour which can be different along the superior–inferior (vertical) direction than in the temporal–nasal (horizontal) direction, and associated factors as discussed above. To avoid any further damage to the cornea during mechanical surgery, the maximum strains should be maintained within failure limits.
Although, [22] has shown that the error in results, obtained using strip tensile (coupon) tests and trephinate spherical inflation tests, may be reduced by incorporating for various geometrical variations in strip testing stress–strain calculations. However, considering the effect of rate of loading strain, the results obtained may not be linked in case of some medical conditions such as Glaucoma, which sees a significantly slower rate of development of internal pressure to cause stresses over cornea. Therefore, present study is confined to its application towards mechanical surgery.

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