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Effect of Recovery Time on the Viscoelastic Behaviour of the Cornea and Sclera

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Introduction

When characterising biomechanical behaviour of soft biological tissue, it is often desirable to subject the same sample to repeated mechanical tests [1]. However, it is well known that strain history can have a compounding effect on subsequent viscoelastic response of the tissue [2]. Various protocols have been employed by researchers when characterising ocular biomechanics resulting in a wide variation in the reported behaviour. While removing strain history is desirable when the intention is to isolate specific behaviour characteristics, it is also important to quantify its contribution to subsequent behaviour. **In this study, we assess the effect of recovery time on the viscoelastic behaviour of the cornea and sclera using uniaxial tensile testing.**

Methods

One-hundred and twenty-eight porcine eyes (sixty-four pairs) were obtained fresh from a local abattoir and tested within twelve hours post-mortem. Upon arrival at the Biomechanics Lab, all extraocular tissues (eyelids, orbital fat, muscles) were removed and the left and right eyes were anatomically orientated based on the location of the optic nerve head (Figure 1). In order to facilitate repeatable removal of tissue strips from the same location and orientation in each eye, a gentian violet pen (Z-SS-665, Schuco International, UK) was used to mark the sclera posterior pole and superior-inferior points at the corneoscleral limbus. A line was then drawn between the three points to mark the superior-inferior meridian (Figure 1).

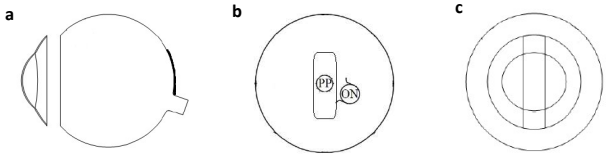
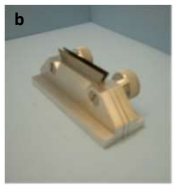


*Figure 1. Schematic of specimen enucleation method for corneal and scleral strips (**a**) temporal view (**b**) posterior pole view (**c**) anterior view*



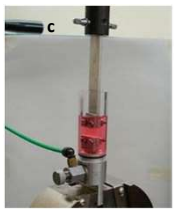


*Figure 2: Instron 3366 (**a**) Bespoke strip cutting tool (**b**) Sample submerged in dextran solution (**c**)*

The strips were then excised according to the schematic shown in Figure 1 and subsequently mounted on a specially designed uniaxial clamping stage and assessed using an Instron 3366 uniaxial testing machine (Figure 2). All strips were subjected to cyclic loading to condition the material before moving to either strain rate sensitivity or stress relaxation tests. Recovery times of 0, 3, 6, and 12 minutes were introduced between the conditioning phase and each of strain rate or stress relaxation phase as shown in (Figure 3).

A number of approaches were used to reduce the possibility of swelling before and during tests:

1. Upon removing the cornea from a given eye, the relevant tissue (cornea or sclera) was stored in its aqueous and vitreous at 4°C and tested at room temperature;
2. Strips were not removed from the cornea or sclera until shortly before testing;
3. All samples were submerged in a 2% w/v Dextran/PBS solution during testing.

The average post mortem time to test was 5±3 hours of death and the testing order was randomised to remove possible effects of tissue degradation[3].

All samples were then subjected to 10 preconditioning cycles between 0.01 and 0.25N before moving to either increasing strain rate or stress relaxation tests. Strain rates were applied over three orders of magnitude (1%, 10% and 100%min^{-1}) whilst stress relaxation was assessed at increasing ramps (0.03, 0.06, 0.09, 0.12 and 0.25N) with a set recovery time (0, 3, 6 or 12 minutes) included between each strain rate or stress relaxation test phase. Load and elongation data were then analysed to derive the tissue's behaviour using a bespoke MATLAB script.

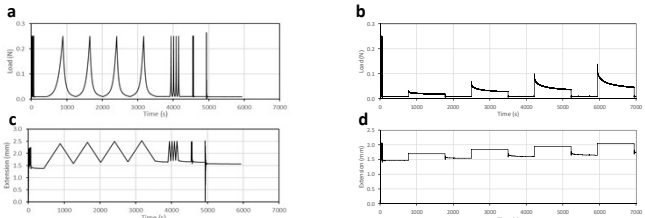


*Figure 3: Load [N] Vs Time [S] for observation of strain rate sensitivity (**a**), stress relaxation (**b**) Extension [mm] vs Time [S] for strain rate sensitivity (**c**) and stress relaxation (**d**)*

Results

Figure 4 shows tangential stiffness for increasing levels of stress. Stress within the samples was calculated using dimensions recorded subsequent to the dissection process. Figure 5 shows normalised stress values of samples calculated as a percentage of the overall stress remaining in the tissue in reference to its stress at the start of the relaxation period. Of 64 corneal samples tested the mean thickness was reported as 0.8458mm (±0.0907mm), a mean width of 4.434 (±0.237mm) and a mean length of 13.179mm (±0.866mm). For 64 scleral samples the mean thickness was 0.9817mm (±0.1365mm), a mean width of 4.240mm (±0.276mm) and a mean length 12.480mm (±0.667mm). Specific samples could only be subjected to either the rate or relaxation protocol and for a specific recovery period, and as such, Figure 4 and Figure 5 show the variation of tangential stress and relaxation behaviour respectively for 8 samples.

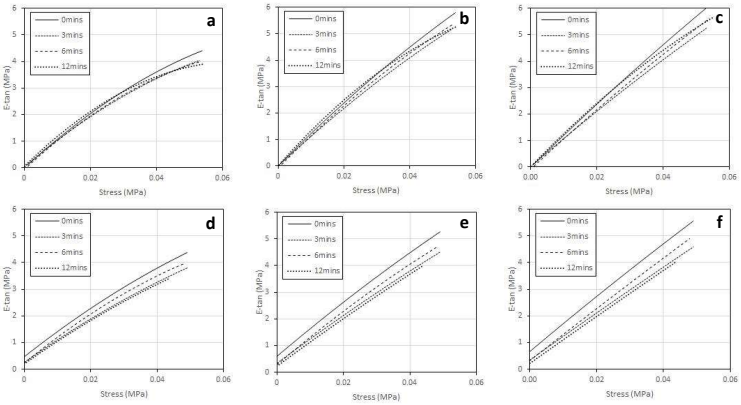


*Figure 4: Tangential stress (MPa) Vs Stress (MPa) for loading rates of corneal samples at 1%/min (**a**), 10%/min (**b**), and 100%/min (**c**) and for scleral samples at 1%/min (**d**), 10%/min (**e**) and 100%/min (**f**)*

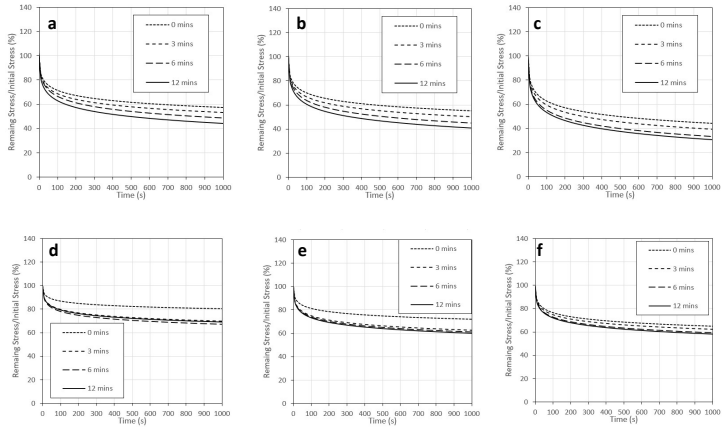


*Figure 5: Normalised stress (%) Vs time (MPa) for initial relaxation loading condtions of corneal samples at 0.09N (**a**), 0.12N (**b**), and 0.25 (**c**) and for scleral samples at 0.09N (**d**), 0.12N (**e**) and 0.25N (**f**)*

Figure 4 demonstrated an increased tangential stiffness for an equivalent stress when no recovery period was imposed, for all rates of loading, in both corneal and scleral samples. Variation in behaviour was more apparent in scleral samples (**d**, **e** & **f**). Figure 5 showed a decreased normalised stress for equivalent values of time when increasing the recovery period for corneal samples (**a, b** & **c)** Whereas scleral samples displayed significantly higher normalised stress when samples were subjected to no recovery time between loading.

Discussion and Conclusion

In both instances stress levels imparted on samples were selected due to their physiological relevance. With lower stresses in testing this was equivalent typical internal ocular pressure (IOP). Increasing values of stress demonstrated behaviour at glaucomatous IOPs and the largest stress values samples were subjected to were equivalent to eye rubbing force often related to Keratoconus. With both strain rate and stress relaxation behaviour little variation was observed between recovery periods at these physiologically relevant stresses provided a recovery period of at least 3 minutes was implemented in the testing protocol.

References

1. Elsheikh A, Kassem W, Jones SW. Acta Bioeng Biomech. 2011;13(2):25-36.
2. Carew EO, Barber JE, Vesely I. Ann Biomed Eng. 2000;28(9):1093-100.
3. Grover CN, Cameron RE, Best SM. J Mech Behav Biomed Mater 2012 Jun;10:62-74.