1 # Inflation Experiments and Inverse Finite Element Modelling of Posterior Human
2 # Sclera
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29 **Contributions:**

30 A. Elsheikh designed and supervised the project. PR provided donor specimens. BG carried

31 out the experiments, analysed the data, interpreted results and wrote the manuscript. WK

32 analysed the results. AA analysed the results and edited the manuscript. RA, SJ and A. Eliasy

33 interpreted the results and edited the manuscript. All authors have reviewed the manuscript,

34 approved the final draft and provided a significant contribution to the study.

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38 **Abstract**

39 The complexity of inverse finite element modelling methods used in ocular biomechanics

40 research has significantly increased in recent years in order to produce material parameters

41 that capture microscale tissue behaviour. This study presents a more accessible method for

42 researchers to optimise sclera material parameters for use in finite element studies where

43 macroscale sclera displacements are required.

44

45 Five human donor sclerae aged between 36 and 72 years were subjected to cycles of internal

46 pressure up to 61mmHg using a custom-built inflation rig. Displacements were measured

47 using a laser beam and two cameras through a digital image correlation algorithm. Specimen-

48 specific finite element models incorporating regional thickness variation and sclera surface

49 topography were divided into six circumferential regions. An inverse finite element procedure

50 was used to optimise Ogden material parameters for each region.

51

52 The maximum root mean squared (RMS) error between the numerical and experimental

53 displacements within individual specimens was 17.5 µm. The optimised material parameters

54 indicate a gradual reduction in material stiffness (as measured by the tangent modulus) from

55 the equator to the posterior region at low-stress levels up to 0.005 MPa. The variation in

56 stiffness between adjacent regions became gradually less apparent and statistically

57 insignificant at higher stresses.

58

59 The study demonstrated how inflation testing combined with inverse modelling could be used

60 to effectively characterise regional material properties capable of reproducing global sclera

61 displacements. The material properties were found to vary between specimens, and it is

62 expected that age could be a contributing factor behind this variation.

63

Introduction

Finite element (FE) modelling has long been recognised as a valuable tool for better understanding ocular response to mechanical actions. Many early studies focused on the cornea, where FE modelling was used to simulate the conditions caused by intraocular pressure (IOP) elevation or experienced in tonometry (Elsheikh and Wang, 2007), impact (Uchio et al., 1999), surgery (Alastrue et al., 2006, Fernandez et al., 2006) and disease (Gefen et al., 2009). Similarly, the behaviour of the sclera, the optic nerve head (ONH) and lamina cribrosa was the subject of several simulation studies due to their importance in the development and progression of glaucoma (Coudrillier et al., 2013, Downs et al., 2003, Eilaghi et al., 2010, Girard et al., 2009b, Girard et al., 2011, Grytz et al., 2014a, Sigal, 2009, Sigal et al., 2005).

In order to pursue the use of FE modelling as a tool in ophthalmology-related research, the ability to experimentally characterise the biomechanical behaviour of ocular tissues is of primary importance. Of the various test methods used, inflation is considered the most desirable owing to the similarity in loading mode experienced by the tissue when compared to in vivo intraocular pressure. Due to its anisotropic behaviour, complex geometry and variable wall thickness, inverse FE modelling has become an increasingly common method of determining sclera material properties. Since its first application in the field of ocular biomechanics by Woo et al. (1972), the complexity of inverse FE modelling methods have significantly increased. For instance, Girard et al. (2009c) developed an anisotropic hyperelastic constitutive model that predicted preferred collagen fibre orientation. Grytz and Meschke (2010) applied a method that also accounted for collagen crimp while Coudrillier et al. (2013) and Zhou et al. (2019) incorporated wide-angle x-ray scattering (WAXS) data of collagen fibril orientations. These intricate approaches allow for the characterisation of microscale behaviour of the sclera. However, implementation of the necessary processes can

90   be time consuming and the incorporation of collagen distribution data into an FE model

91   requires technical user subroutines that are not always accessible to researchers.

92

93   This study aimed to produce a set of regional Ogden material parameters capable of predicting

94   macroscale deformation behaviour of the sclera. Ex-vivo specimens were tested using a

95   combination of experimental inflation, 2D digital image correlation (DIC) and inverse FE

96   analysis. Specimen-specific FE meshes divided into six circumferential regions, each with an

97   optimised pair of parameters capable of reproducing macroscale sclera displacements, were

98   used in the inverse analysis. This approach accounts for the previously reported anterior to

99   posterior variation in sclera material properties without the need to include detailed

100   microstructure data thereby reducing the time required for data acquisition and post-test

101   analysis. The presented methodology, and its results, can be used in FE studies where

102   macroscale sclera displacements are apt.

103

Materials and Methods

Specimen Preparation

106   Five human donor sclerae were obtained fresh from the Fondazione Banca degli Occhi del

107   Veneto, Italy. The average and standard deviation of the donors' age was 61.8±15.6 years

108   (range 36 to 72 years). Routine screening was used to exclude donors with human

109   immunodeficiency virus, hepatitis B and C, syphilis, central nervous system degenerative

110   diseases, active infections, diabetes, eye tumours and glaucoma. The sclerae were not frozen

111   at any stage. Ethical approval to use the specimens in research was obtained by the eye bank

112   in accordance with the Declaration of Helsinki and its revisions up to 2013. The specimens

113   were extracted approximately 6 hours after death, preserved in storage medium Eusol-C

114   (Alchimia, Padova, Italy) and tested within 3 days post mortem. The limit of 3 days was based

115   on the results of a previous experimental study confirming the maintenance of scleral tissue

116   quality in the preservation medium Eusol-C for up to 5 days (Geraghty et al., 2012).

117

118 The sclerae were surgically detached from the cornea, extraocular muscles, retina and

119 choroid. In order to assess if tissue swelling occurred during transit, nine thickness

120 measurements were obtained between the anterior foramen and posterior pole post-

121 enucleation by the eye bank using an UP-1000 ultrasound pachymeter (Nidek, Gamagori,

122 Japan). The superior cardinal point on the anterior foramen edge and the measurement

123 locations were marked using a gentian violet pen, as shown in Figure 1 (a) and (b). The

124 measurements were repeated at the same locations before conducting the tests at the authors'

125 laboratory using a Pachmate 55 (DGH Technologies, Exton, PA) with 5 μm accuracy. The

126 average difference between the two sets of measurements was 4±25 μm, which is within the

127 range previously reported for a comparison of conventional and handheld pachymeters

128 (Queirós et al., 2007), thereby confirming that tissue swelling did not occur. The sclerae

129 dimensions were measured using an electronic Vernier calliper (D00352, Duratool, Taiwan)

130 with 10μm accuracy. The diameter of the anterior foramen after removal of the cornea was

131 17.75±0.64 mm, the specimen depth from the foramen edge to the posterior pole 19.54±0.45

132 mm and the equatorial diameter 24.26±0.38 mm, as shown in Figure 1 (b).

133

134 The superior, inferior, temporal and nasal (orthogonal) directions and the four 45° and 135°

135 (diagonal) directions were marked on the anterior foramen edge of each specimen. The

136 posterior pole was located by placing the specimens on their anterior foramen and marking

137 the uppermost point on the posterior region. A flexible plastic strip marked with 2 mm

138 increments was extended between the posterior pole and the anterior foramen to provide a

139 guide for thickness measurements at 2 mm intervals along the eight meridians shown in Figure

140 1 (c) and (d). The pachymeter obtained 50 measurements per point and the average value

141 was recorded. The regional thickness variation data obtained from this procedure was utilised

142 in the generation of specimen-specific finite element meshes for the inverse analysis

143 procedure.

144

Inflation testing

145

146   The sclerae were anatomically orientated and fixed along their anterior foramen using a

147   specially designed clamping mechanism. The clamps provided a secure grip of the anterior

148   sclera without the need for adhesives, as shown in Figure 2 (a). The clamped sclerae were

149   filled with a saline solution before connection to an inflation test rig which was developed in

150   an earlier study (Elsheikh et al., 2007). The specimens had a thin covering of the preservation

151   medium Eusol-C upon removal from storage which prevented dehydration of the outer surface

152   during testing.

153

154   Each specimen was subject to three cycles of internal pressure change from 1 mmHg up to

155   61 mmHg. Cyclic pressure changes were managed by means of a small reservoir whose

156   vertical movement was computer-controlled and set to a pressure change rate of 37.5

157   mmHg/min. The pressure within the sclera was monitored using a differential pressure

158   transducer (FDW, RDP Electronics, Wolverhampton, UK). Initial trials were carried out to

159   assess the number of cycles required to produce repeatable pressure-displacement behaviour

160   and the resulting tissue stiffness at maximum IOP. A significant difference was observed

161   between the first and second cycle but little difference was observed thereafter. Consequently,

162   the results of the third cycle were considered representative of stable material behaviour.

163   Similar to previous studies on the mechanical properties of the sclera (Coudrillier et al., 2012,

164   Fazio et al., 2012), all tests were carried out at room temperature (21°C).

165

Specimen Imaging

166

167   Displacement of the specimens was monitored along the orthogonal (superior-inferior and

168   temporal-nasal) meridians of the sclera using two 14.7 MP digital cameras (Canon, Tokyo,

169   Japan) with 4416 × 3312 pixels per image and orientated as shown in Figure 2 (b). Specimens

170   were connected to the inflation rig ensuring alignment of the temporal-nasal and superior-

171   inferior axes with the orthogonal planes. Prior to testing, a pulverised fuel ash powder was

172   dispersed over the external surface of each sclera to enhance optical contrast. During the third

173   loading cycle, individual images with an accuracy of 12.6µm/pixel were taken at pressures of

174   1, 16, 31, 46 and 61mmHg using Remote Capture software (Canon, Tokyo, Japan).

175

176   2D digital image correlation (DIC) software (geoPIV8, Cambridge, UK) was used to analyse

177   successive camera images and determine the movement of points on the sclera surface in

178   the form of 2D displacement vectors measured in pixels. 1D posterior displacements along

179   the ocular longitudinal axis were also continuously monitored using an LK-031 laser

180   displacement sensor (Keyence, Milton Keynes, UK) with 1 µm accuracy. The laser

181   displacement sensor measurements provided a means of calibrating the DIC software outputs

182   to convert the displacements from pixels to millimetres. The resulting vectors obtained from

183   the 2D DIC analysis were then used to produce pressure-displacement target curves for use

184   in the inverse modelling procedure at the nine points located at 0°, 30° and 60° relative to the

185   longitudinal axis on the superior-inferior and temporal-nasal meridians, as shown in Figure 2

186   (c) and (d).

187

188   **Inverse Modelling Procedure**

189   Sclera material behaviour parameters were derived from the experimental data using HEEDS

190   Professional 5.2 (Red Cedar Technology, Michigan, USA), a design optimisation software, in

191   conjunction with the nonlinear FE software Abaqus (Dassault Systèmes Simulia Corp., Rhode

192   Island, USA). The SHERPA robust optimisation search algorithm, which is embedded in the

193   optimisation software, was used. This algorithm spanned the parameter space while

194   attempting to achieve convergence in targeted regions.

195

196   The FE mesh was generated using a custom written Visual Basic code (Microsoft, Redmond,

197   WA) which utilised a diamatic dome configuration and consisted of one layer of 19661 fifteen-

198   node C3D15H hybrid continuum elements. The FE meshes incorporated specimen-specific

199   outer surface topography and sclera wall thickness variations. Due to the inability to measure

200   the dimensions of the lamina cribrosa, this component was modelled as a group of six

201 elements occupying a circular area with 0.9 mm radius, 0.3 mm thickness, 0.3 MPa Young's

202 modulus and centre located 2.7 mm away from the posterior pole (Saude, 1993, Sigal, 2009).

203

204 Movement of model nodes located on the anterior foramen was restrained in the three main

205 directions, u, v and w, to simulate the conditions created by the mechanical clamps of the

206 inflation rig. All other nodes possessed three degrees of freedom; displacement in u, v and w.

207 Anterior to posterior variation of sclera material behaviour was enabled by dividing the model

208 into 6 regions as illustrated in Figure 3, with region 1 being the most anterior and region 6

209 encompassing the posterior pole. Initial trials found 6 regions to be suitable for the current

210 study as decreasing the number of regions reduced the accuracy of fit between the

211 experimental and numerical results.

212

213 The SHERPA algorithm was used to determine a pair of first-order Ogden material parameters

214 for each sclera region (see Appendix) by minimising the root mean square (RMS_{local}) error

215 between the experimental and numerical posterior displacements at the nine targeted points

216 on the sclera surface using the following objective function for each point where:

$$RMS_{local} = \sqrt{\frac{1}{P} \cdot \sum_{p=1}^{P} (\delta_p^{exp} - \delta_p^{num})^2} \qquad \text{Equation 1}$$

217 where P is the total number of pressure levels at which the RMS is calculated (i.e. 16, 31, 46

218 and 61 mmHg), and δ_p^{exp} and δ_p^{num} represent the orthogonal components of the experimental

219 and numerical displacements for a given point on the sclera surface at pressure level p. The

220 optimisation algorithm also minimised RMS_{total}, the average of the local errors, where:

$$RMS_{total} = \frac{1}{N} \sum_{n=1}^{N} RMS_{local} \qquad \text{Equation 2}$$

221 where N is the total number of selected points on the scleral surface. Nodes in the FE mesh

222 which corresponded to these locations were monitored and the optimisation software adjusted

223   the material parameters of each element group until the best possible fit was achieved

224   between the experimental and numerical pressure-displacement results.

225

226   **Statistical Analysis**

227   The significance of associations between biomechanical properties (tangent modulus) and

228   distance from posterior pole was assessed by Spearman rank correlation. The tests were

229   performed in IBM SPSS Statistics 21 (IBM, Armonk, NY). $P<0.05$ was considered an indication

230   of statistical significance.

231

232   **Results**

233   **Experimental Results**

234   All sclerae exhibited maximum thickness in the region of the posterior pole with values ranging

235   between 1014 and 1108 μm (1076±23 μm). Thickness reduced progressively up to 6mm away

236   from the posterior pole before reducing more rapidly down to a minimum of between 478 and

237   770 μm (630±76 μm) at 20 mm from the posterior pole. This was then followed by a rapid

238   increase that continued to the edge of the anterior foramen (757±59 μm). Beyond 4-6 mm

239   from the pole, measurements taken along the diagonal meridians exhibited greater thickness

240   than the orthogonal meridians, however, all lines followed the same thickness variation

241   pattern. These values were used to produce contour maps of the thickness variation over a

242   developed scleral surface and the average of all specimens is shown in Figure 4. The maps

243   were realised by creating a Delaunay triangulation between the thickness measurement

244   locations and applying a cubic interpolation function within these regions.

245

246   Pressure-displacement behaviour for an example specimen is plotted in Figure 5 (a) and gives

247   a direct comparison of the change in behaviour over three cycles. All specimens exhibited

248   nonlinear pressure-displacement behaviour up to a pressure of between 20 and 30 mmHg.

249   Beyond this level of internal pressure, the behaviour became almost linear. Progression from

250 the first to second loading cycle exhibited a notable stiffness increase in all specimens of

251 34±10% on average over the pressure range. A similar comparison between the second and

252 third cycles showed that the stiffness increase reduced to 5±4%. Initial trials involving four

253 loading cycles yielded only a further 3±4% stiffness increase between the third and fourth

254 cycles, which justified the use of third cycle results as representative of the specimens'

255 repeatable behaviour. Displacements at the posterior pole for all specimens during the third

256 loading cycle ranged between 46 and 126 µm (90±26 µm) at 16 mmHg up to between 116

257 and 220 µm (191±54 µm) at 61 mmHg. Average behaviour observed during the third loading

258 cycle for all specimens is shown in Figure 5 (b).

259

260 Analysis of specimen deformation using DIC demonstrated a gradual reduction in posterior

261 displacement towards the equatorial region from a maximum at the posterior pole, Figure 6.

262 At the maximum pressure applied, displacement of the transverse plane (temporal-nasal

263 meridian) points positioned at 30° and 60° relative to the longitudinal axis were 155±15µm and

264 113±17µm, respectively. The corresponding points on the sagittal plane (superior-inferior)

265 meridian experienced similar displacements of 160±15µm and 118±19µm.

266

267 **Numerical Results**

268 Following optimisation, the FE models demonstrated the same progressive increase in

269 posterior displacement that was evident in the specimens during the experimental stage of the

270 study, Figure 7. An example comparison of experimental displacements at the 9 points on

271 Specimen 5 at which displacements were monitored, and those produced by the

272 corresponding numerical model is shown in Figure 8. The comparison shows that model

273 predictions closely matched the experimental results. The average errors for all specimens at

274 the 9 points on the sclera when IOP was equal to 16, 31, 46 and 61mmHg were 8±7µm,

275 8±7µm, 10±8µm, and14±10µm, respectively.

276

277 The material parameters obtained for all specimens (see Table 1 in Appendix) were used to

278 enable comparisons between the tangent modulus values, as calculated from circumferential

279 stress-strain behaviour, in different regions relative to those around the posterior pole, Figure

280 9. The comparisons were held at four stress levels; 0.001, 0.005, 0.01 and 0.1 MPa, and it

281 was clear that under low stresses (up to 0.005 MPa) there was a gradual reduction in tissue

282 stiffness towards the posterior pole. For three of the five specimens, this reduction in stiffness

283 was statistically significant at the 0.01 level. However, as the stress levels increased, the

284 differences in stiffness between the six regions became gradually less apparent and

285 statistically insignificant. The average circumferential stress-strain and tangent modulus-

286 stress behaviour over the six regions for all specimens is presented in Figure 10.

287

288 **Discussion**

289 This study presents a novel method of optimising regional material properties capable of

290 replicating global displacements, as measured at nine target points, from experimental

291 inflation testing of human sclerae using 2D DIC and inverse FE modelling. Specimen-specific

292 FE meshes incorporating regional thickness and topography variations were constructed

293 using elements divided into six circumferential regions. Each region was assigned a first-order

294 Ogden constitutive material model, the parameters of which were optimised in order to

295 minimise RMS errors of pressure-displacement behaviour at locations in the FE models that

296 corresponded to the nine target points on the sclera surface. The maximum RMS error

297 between the experimental and numerical displacements within individual specimens was

298 17.5μm, demonstrating the ability of this method to obtain material behaviour parameters for

299 ophthalmology-related FE research studies where macroscale sclera displacements are

300 required.

301

302 The experimental protocol subjected human sclerae to cycles of internal pressure up to 61

303 mmHg to assess global displacements beyond the normal physiological and elevated

304 pressure ranges typically seen in glaucoma. The pressure-displacement behaviour observed

305 during the inflation tests closely matched the nonlinear behaviour observed in similar testing

306 scenarios for human (Fazio et al., 2012, Pyne et al., 2014, Tang et al., 2013, Woo et al., 1972),

307 monkey (Girard et al., 2009a, Girard et al., 2009b) and porcine (Girard et al., 2008) sclera.

308 Variations in pressure-displacement behaviour observed between different specimens could

309 be partly attributed to the age variation between donor specimens used in this study

310 (Coudrillier et al., 2015a).

311

312 All specimens demonstrated considerable thickness variation with maximum values in the

313 vicinity of the posterior pole, reducing to a minimum close to the equator before increasing

314 towards the anterior foramen. Similar to earlier studies (Elsheikh et al., 2010, Norman et al.,

315 2010), measurements along the orthogonal meridians demonstrated reduced thickness

316 compared to the diagonal meridians, however, all lines followed the same overall trend. The

317 biomechanical behaviour results show a consistent trend of gradual stiffness reduction from

318 the equator towards the posterior pole. While the trend was clear and statistically significant

319 (P=0.01) for the majority of specimens at low stresses up to 0.005 MPa, it became less

320 apparent and statistically insignificant under larger stresses for all specimens.

321

322 Various approaches have been used in inverse FE analyses of the posterior sclera. In a study

323 carried out by Woo et al. (1972), the sclera was modelled as a single set of isotropic elements

324 and their material behaviour was adjusted to fit a trilinear behaviour curve. In the work of Girard

325 et al. (2009c), the posterior sclera was divided into nine sub-regions. Four sub-regions were

326 orientated around the peripheral sclera, four around the parapapillary sclera and one around

327 the optic disc and preferred fibre orientations were optimised for each region. In the work of

328 Coudrillier et al. (2013), particular attention was given to the region spanning from the optic

329 nerve to the mid-peripheral sclera where specimen-specific information on collagen

330 orientations was obtained using WAXS. DIC-measured displacements were then applied as

331 kinematic boundary conditions around this region in their models. A more recent study by

332 Kollech et al. (2019) applied a different approach where sub-regions of the sclera were defined

333   based on first principal strains, as measured using sequential DIC. Inverse FE analysis was

334   then used to optimise Holzapfel anisotropic material parameters within each sub-region.

335

336   The aforementioned advances in ophthalmology-related inverse FE modelling have provided

337   valuable insights into the effects of scleral anisotropy (Coudrillier et al., 2013, Girard et al.,

338   2009b, Grytz et al., 2014a), age-related stiffening (Coudrillier et al., 2015a, Coudrillier et al.,

339   2012, Girard et al., 2009a), diabetes (Coudrillier et al., 2015b), race (Grytz et al., 2014b) and

340   chronic IOP elevation (Coudrillier et al., 2015c, Girard et al., 2011). The inclusion anisotropic

341   material properties are undoubtedly necessary for FE studies that aim to investigate the

342   mechanical response of the optic nerve head. While variations in the mechanical properties of

343   the lamina cribrosa have been shown to have little effect on the surrounding sclera (Girard et

344   al., 2009b), the removal of anisotropy from the peripapillary region can significantly affect the

345   behaviour of the sclera canal and lamina cribrosa (Coudrillier et al., 2013). However, in studies

346   where microscale sclera displacements are not required such as design optimisation of

347   ophthalmology-related medical devices or corneal studies where only global sclera

348   displacement behaviour is important, our methodology can be applied to obtain suitable

349   material parameters for use in FE models.

350

351   There are a number of limitations related to this study. Firstly, the same clamping mechanism

352   was used in all inflation tests. In addition to the fixed boundary conditions created by the

353   clamps, the use of a single clamp size coupled with the inter-specimen variation in sclera

354   diameter (24.26±0.38 mm) would be expected to induce non-physiological stresses within the

355   adjacent tissue. While this impacted the overall posterior displacement of the sclera, a trial

356   carried out using FE analysis found the fixed boundary effects within the tissue to have

357   diminished by the specimen equator.

358

359   Secondly, the FE model was based on a number of assumptions which could have affected

360   the accuracy of its predictions. The division of the sclera into circumferential regions, although

361   justified by the approximately circumferential orientation of the displacement contour lines

362   (Figure 6), ignored possible variations in the scleral microstructure. A related limitation is the

363   assumption of isotropic sclera material behaviour in the FE models. This assumption, which

364   was necessary because of the same lack of information on scleral microstructure, was based

365   on a study in which the posterior sclera behaviour was found to be almost isotropic with the

366   average difference in stiffness between two orthogonal loading directions limited to 6%.

367

368   Thirdly, target curves used in the inverse modelling procedure were obtained from points along

369   the orthogonal meridians only. Consequently, and in contrast to studies that used fibre

370   reinforced models (Coudrillier et al., 2013, Coudrillier et al., 2012, Grytz et al., 2014a, Pyne et

371   al., 2014), the optimised material parameters presented in this study provide a means of

372   simulating whole hemisphere behaviour but may not represent more local behaviour at points

373   on the sclera shell between the meridians. Similarly, the finite element models were based on

374   the thickness measurements along the orthogonal and diagonal meridian lines, and

375   interpolation of thickness values was necessary for the areas between the meridian lines. It is

376   difficult to estimate the effect of this approximation on the model's predictions, but it is unlikely

377   to be significant. A further approximation was the simple and standard form assumed for the

378   lamina cribrosa, which was necessary due to our inability to distinguish between the nerve

379   fibres and scleral tissue and to measure the dimensions of the lamina cribrosa. While these

380   limitations, combined, are expected to have an effect on the FE predictions of the distribution

381   of stress, strain and deformation, they are unlikely to have a notable effect on overall

382   behaviour.

383

384   Finally, the loading regime adopted in this study was similar to those followed by other

385   researchers (Danielsen, 2004, Girard et al., 2009a, Schultz et al., 2008, Zeng et al., 2001)

386   where no recovery was included between the conditioning cycles and the specimens were not

387   allowed to creep before the final loading cycle. However, it is recognised that other studies

388   introduced different loading regimes incorporating recovery and creep and this could have an

389   effect on the obtained behaviour (Boyce et al., 2008). In particular, it has been argued that

390   without recovery time the behaviour is likely to be affected by the strain history of

391   preconditioning cycles (Carew et al., 2000). In appreciation of the potential importance of these

392   effects, further tests will be conducted in which variations in the loading regime, including

393   protocols to assess viscoelasticity, will be introduced to assess their effect on the obtained

394   behaviour.

395

396   **Conclusion**

397   The study demonstrated how inflation testing combined with inverse modelling could be used

398   to effectively characterise regional material properties of ocular tissue using circumferential

399   regions of isotropic elements to replicate macroscale sclera displacements. The material

400   properties were found to vary between specimens, and it is expected that age could be a

401   contributing factor behind this variation. While this study laid out the experimental and inverse

402   modelling procedures and presented overall behaviour patterns, follow up studies will attempt

403   to characterise the variation in properties of scleral tissue with age and medical history.

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518

519

Figure Captions

520

521   Figure 1 (a) and (b) Schematic elevations of a sclera including (a) a view of specimen

522   orientation from the anterior side after cornea removal, and (b) a view of specimen dimensions

523   from the temporal side. Circular points = approximate locations of eye bank thickness

524   measurements. (c) and (d) Schematic elevations of sclera wall thickness measurement

525   locations obtained along eight meridians lines between the anterior foramen and posterior

526   pole, including (c) a view from the temporal side, and (d) a view from the posterior side. PP =

527   Posterior Pole, ON = Optic Nerve.

528

529   Figure 2 (a) Temporal view of a schematic cross-section through a clamped sclera showing

530   direction of the laser displacement sensor, and (b) posterior view of the sclera showing the

531   camera positions whereby camera 1 monitors the temporal-nasal meridian and camera 2

532   monitors the superior-inferior meridian. (c) and (d) Schematic elevations of sclera illustrating

533   the numbered points at which posterior displacements were monitored, including (c) a view

534   from the temporal side, and (d) a view from the posterior side. PP = Posterior Pole. ON = Optic

535   Nerve.

536

537   Figure 3 (a) Nasal and (b) posterior illustration of the finite element mesh showing the six

538   element groups used during the inverse modelling procedure. ONH = Optic Nerve Head

539

540   Figure 4 Contour map of thickness variation at 25 μm intervals on a developed scleral surface

541   for (a) average data obtained from all sclerae tested, and (b) represents the locations of

542   thickness measurements. Colour bar values are presented in micrometres (μm). The map

543   centre represents the posterior pole. Maps continue to the anterior foramen edge. S = superior

544   direction, I = inferior direction, N = nasal direction, T = temporal direction, ONH = optic nerve

545   head.

546   Figure 5 Example pressure-displacement behaviour measured at the posterior pole for (a) a

547   58 year old donor specimen over three cycles (plotted from a point of zero displacement to

548   allow direct comparison between cycles) and (b) average pressure-displacement behaviour

549   during the third loading for all specimens.

550

551   Figure 6 Contour maps of posterior displacements in millimetres observed experimentally from

552   camera 1 (superior view) for a 58 year old donor specimen.

553

554   Figure 7 Example of FE model-predicted posterior displacements in millimetres over the sclera

555   surface for a 58 year old donor specimen. Reduced thickness section on left side of model

556   represents the location of the lamina cribrosa.

557

558   Figure 8 Example of fit between experimental and numerical posterior displacements for a 58

559   year old donor (Specimen 5), obtained at the monitored points shown in Figure 2 (c) and (d)

560   on the sclera surface.

561

562   Figure 9 Ratios for tangent modulus within each region (R1 to R6) of the sclera models relative

563   to tangent modulus at the posterior pole (R6) when stress is equal to (a) 0.001 MPa, (b) 0.005

564   MPa, (c) 0.01 MPa, and (d) 0.1 MPa.

565

566   Figure 10 Average behaviour in the circumferential direction for each sclera showing (a)

567   stress-strain and (b) tangent modulus-stress trends for the six regions. Error bars represent
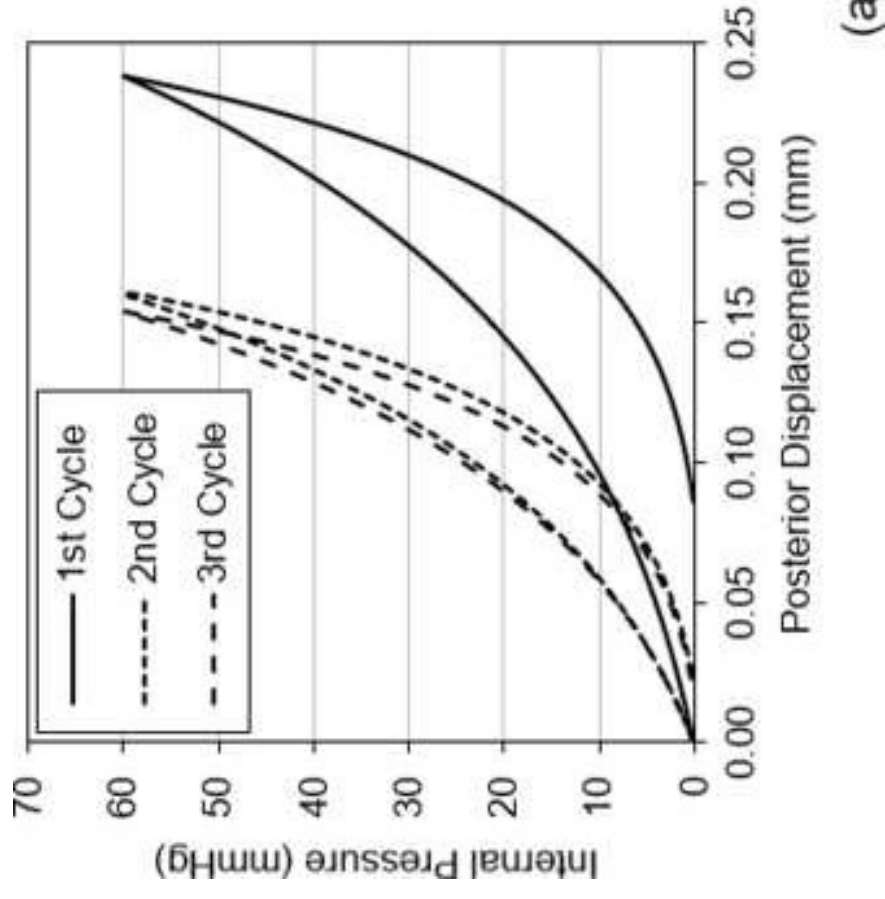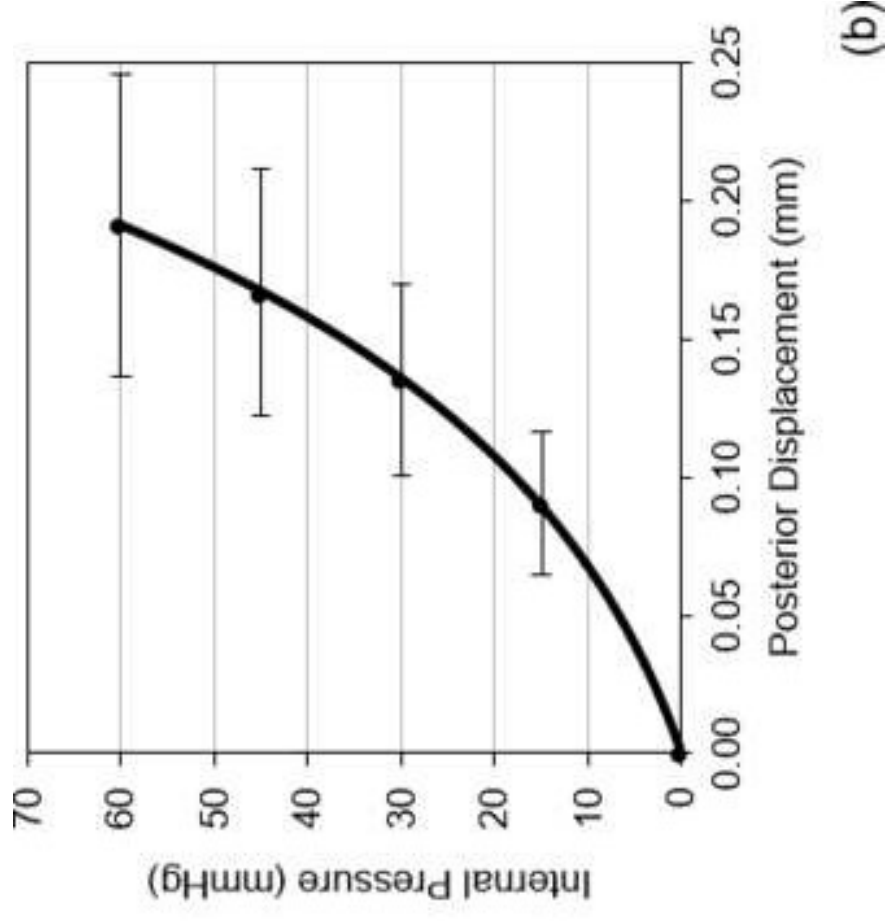
568   standard deviation.

(a)

(b)

(c)

(d)

Figure_2

(a)

(b)

(c)

(d)

Figure_3

(b)

(a)

Figure_4

(a)

(b)

Figure_5

Figure_6

Figure_7

16mmHg

31mmHg

46mmHg

61mmHg

Figure_8

Figure_9

Figure_10

(a)

(b)