



In vivo measures of anterior scleral resistance in humans with rebound tonometry

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Abstract

Purpose: To measure regional variations in anterior scleral resistance (ASR) using a ballistic rebound tonometer (RBT) and examine whether the variations are significantly affected by ethnicity and refractive error (RE).

Methods: ASR was measured using a RBT (iCare TA01) following calibration against the biomechanical properties of agarose biogels. Eight scleral regions (nasal, temporal, superior, inferior, inferior-nasal, inferior-temporal, superior-nasal and superior-temporal) were measured at locations 4mm from the limbus. Subjects were 130 young adults comprising three ethnic groups whose RE distributions [MSE (D) \pm S.D.] incorporated individuals categorised as without-myopia (NM; MSE \geq -0.50) and with-myopia (WM; MSE $<$ -0.50); British-White (BW): 26 NM + 0.52 \pm 1.15D; 22 WM -3.83 \pm 2.89D]; British-South-Asian (BSA): [9 NM + 0.49 \pm 1.06D; 11 WM -5.07 \pm 3.76D; Hong-Kong-Chinese (HKC): [11 NM + 0.39 \pm 0.66D; 49 WM -4.46 \pm 2.70D]. Biometric data were compiled using cycloplegic open-field autorefraction and the Zeiss IOLMaster. Two- and three-way repeated measures analysis of variances (ANOVAs) tested regional differences for RBT values across both refractive status and ethnicity whilst stepwise forward multiple linear regression was used as an exploratory test. **Results:** Significant regional variations in ASR were identified for the BW, BSA and HKC ($p < 0.001$) individuals; superior-temporal region showed the lowest levels of resistance whilst the inferior-nasal region the highest. Compared to the BW and BSA groups, the HKC subjects displayed a significant increase in mean resistance for each respective region ($p < 0.001$). With the exception of the inferior region, ethnicity was found to be the chief predictor for variation in the scleral RBT values for all other regions. Mean RE group differences were insignificant.

Conclusions: The novel application of RBT to the anterior sclera confirm regional variation in ASR. Greater ASR amongst the HKC group than the BW and BSA individuals suggests that ethnic differences in anterior scleral biomechanics may exist.

Introduction

Owing to its role in the aetiology of various ocular pathologies, there is growing interest in assessing and understanding the material and biomechanical attributes of the sclera. Challenges in isolating the mechanical resistance offered by the sclera from the intraocular pressure (IOP) and the

surrounding tissues, has made characterising these properties in the living eye notoriously complex. Data from *in vitro* experiments based on extensometry^{1,2}, globe inflation testing^{3,4} as well as finite element modelling⁵⁻⁷ provide much of the evidence in the literature. Whilst these findings have no doubt improved our understanding of scleral biomechanics, as yet, none of these methods are suitable

for the clinical assessment of the *in vivo* human sclera. Since scleral structural changes that accompany pathology are augmented across the posterior segment, most studies are based upon assessing these more discernible alterations. Although the anterior sclera is more accessible, technical limitations in assessing it has meant there is uncertainty as to its role in the pathogenesis of the various eye diseases. Tentative efforts to assess *in vivo* anterior scleral properties^{8–11} suggest that it displays regional variation in shape,¹² thickness^{10,13,14} and resistance.⁹ Application of indentation tonometry to the anterior sclera has been shown to be a robust method of assessing its gross mechanical resistance; however, the contact nature of the procedure as well as the need for topical anaesthesia presents limitations. As such, rebound tonometry (RBT) may provide a possible alternative. RBT determines the IOP by assessing the ballistic properties of a probe on rebound from the eye.¹⁵ When applied to the cornea, the IOP and viscoelastic properties of the cornea are the key determinants of the characteristics of the rebound response.^{16–21}

The present investigation examined the utility and validity of the RBT as a surrogate for assessing anterior scleral resistance. To interpret the scleral RBT values as measures of scleral resistance the study describes a calibration exercise using agarose biogels of varying rigidity. To confirm previous observations of regional variation in scleral resistance and to assess whether such differences vary with ethnicity^{22,23} and refractive status,²⁴ the study examines measures of anterior scleral resistance in individuals with and without myopia of Hong-Kong Chinese, British South Asian and British-White descent.

Methods

Calibration of the rebound tonometer with agarose gels of different stiffness

Preparation of agarose gels

The technique for assessing agarose rigidity was adapted from the British Pharmacopoeia²⁵ protocol for assessing the biomechanical properties of gelatine. Agarose Molecular Biology Reagent (Moisture < 10; www.mpbio.com) was used to prepare biogels of eight concentrations (i.e. % w/v) in the following order using serial dilution: 2.00, 1.75, 1.50, 1.25, 1.00, 0.75, 0.50, and 0.20; 10 vials of each concentration were made. Visual inspection was conducted to remove vials with any obvious bubbles or non-uniformity of the meniscus surface.

Tensometry on agarose gels

The Hounsfield tensometer (www.tiniusolsen.com) applies a controlled force to a sample of material and produces a force-displacement graph. The tensometer applies the force

at a constant rate and in turn, readings of force and extension are recorded until rupturing of surface tension is evident. A load of up to 5 Newtons and displacement range (mm) of 0–2000 was applied. A rebound tonometer probe was used as the indenter. Each vial was held in a clamp below the indenter probe to aid stability and alignment and only indented once. On press button initiation of the test sequence the machine moves the crosshead down at a constant speed of 2 mm min⁻¹. On detection of a load by the instrument load cell, the force-extension results are graphically displayed on a linked PC monitor. The data were exported via the QMAT (Questions MATerials, www.tiniuolsen.com) graphical software into a Microsoft Excel (www.microsoft.com) spreadsheet. The force-extension graphs for each vial from the Hounsfield tensometer output were initially converted to stress-strain graphs. Data from the loading portion of the stress-strain graphs were used to estimate Young's modulus (E (kilopascals, kPa)). E provides a measure of the stiffness of a material with higher levels of E indicating greater stiffness. In accordance with previous studies the approximately linear portion of the stress-strain curves (i.e. in the 4%–6.5% strain range) was assessed to determine the E values.^{26,27}

Application of RBT to agarose gels

The iCare RBT (TA01, www.icaretonometer.com) device projects a small light-weight probe towards the ocular surface and extrapolates the IOP from the probe's rebound kinetics. The operational principles of the device have been extensively described previously.^{15,28–30} For the purposes of this study the tonometer was table mounted onto a specially constructed movable base allowing the distance to be kept constant throughout the measurement session by locking the instrument in place once the probe was aligned and perpendicular to the biogel. The vials were held horizontally with a retort stand/clamp fixture. Using an electronic calliper (www.maplin.co.uk) the tonometer probe was set at 6 mm from the biogel meniscus. A spirit level was used to ensure each vial was level and the tonometer probe was aligned subjectively to provide central readings. On press button activation, four valid and reliable separate readings (each reading constitutes six measurements) were taken. Repeatability of the agarose RBT readings was assessed by performing the above procedure five times on 10 vials for each of the eight concentrations.

Application of RBT to assess scleral resistance

Subjects were recruited from the staff and student population at Aston University, UK and the Hong Kong Polytechnic University, School of Optometry, Hong Kong. Eligibility to take part in the study was confirmed after subjects completed a screening questionnaire. As the investigation was conducted within a university setting, all subjects

were required to have a full eye examination within the last two years. A slit lamp examination was conducted on all eligible subjects to ensure no active anterior segment abnormalities were present. As ocular biomechanics have been previously shown to be effected by various ocular diseases and conditions, the exclusion criteria included previous history of ocular surgery, trauma or pathology, ocular medication and astigmatism > 1.75 D. Furthermore, individuals suffering from connective tissue related disorders were also excluded due to their accepted effect on collagen composition and hence scleral biomechanics.³¹ One hundred thirty subjects gave written informed consent prior to participating in the study. Subjects were categorized as without-myopia (NM; $MSE \geq -0.50$) or with-myopia (WM; $MSE < -0.50$). Ethical approval was obtained from Aston University and Hong Kong Polytechnic University Ethics Committees and the study was performed according to the tenets of the Declaration of Helsinki.

Procedure for the use of the rebound tonometer on the sclera

The table mounted RBT allowed the eye-probe distance to be kept constant throughout the measurement session by locking the instrument in place once the probe was aligned and perpendicular to the cornea. To minimise head tilt and to further control probe-eye distance in different directions of gaze, subjects were asked to place their head against a forward headrest band; the head was then strapped into a stable position with a rear Velcro belt. The tonometer was aligned with the tip of the probe 4–8 mm from the apex of the cornea in primary gaze. RBT was performed in eight scleral locations: nasal (N), temporal (T), superior (S), inferior (I), inferior nasal (IN), inferior temporal (IT), superior nasal (SN) and superior temporal (ST). To expose the sclera in these different locations the subjects were asked to follow a mobile fixation target and maintain their gaze steady. Keeping the RBT in one position and having the eye rotate in various different gazes ensured the probe was approximately perpendicular to the ocular surface at all times.¹⁸ The scleral RBT readings were taken from approximately 4 mm from the limbus to avoid areas of muscle insertion, which are known to affect scleral microstructure.³² To aid location of the scleral site a custom-designed graticule was attached to the end of the RBT which allowed the examiner to judge distances approximately 4 mm from the limbus where the probe would make contact with the sclera horizontally, vertically, and in the oblique meridians. To avoid any order effects and to minimise the possible effect of initial measurements on subsequent readings, the order of eye examined and the sequence in which the eight regions were assessed was randomised. Two readings (each reading averaged from the six measurements) were taken successively for each gaze position (before the direction of gaze was changed) to reduce the effect of localised massaging of the

sclera. The procedure was repeated until at least four valid readings were recorded for each location on the sclera. To ensure consistency in the data collected from both Aston University and Hong Kong Polytechnic the same table mounted RBT was used throughout the study.

Intra- and inter-observer variation for both corneal and scleral RBT measurements

Intraobserver variation of corneal and scleral RBT measurements was examined by repeating the procedure as described above on five separate occasions on two subjects. A period of four days was left between readings to ensure results were not biased by previous results and the examiner was blind to the data from the previous sessions. Inter-observer variability was evaluated by having two examiners perform RBT on the cornea and sclera of 11 normal subjects. For both intra and inter-observation variation, subjects were seen at the same time of day to control for the effect of diurnal variation in IOP and scleral thickness.^{33,34}

Biometric measurements

Cycloplegia was induced in both eyes using 1 drop of tropicamide HCl 1% (Minims®, www.bausch.co.uk). An objective measure of the refractive error was determined with a binocular open view autorefractor/keratometer (Shin-Nippon SRW-5000, www.shin-nippon.jp). Five measurements were taken from both eyes, averaged and converted to mean spherical error (MSE) (sphere power + $0.5 \times$ cylinder power). Axial length (AL) measurements were acquired using the IOLMaster (www.zeiss.co.uk); five separate measurements were averaged for AL.

Statistical analysis

All statistical analysis was performed using IBM SPSS v. 23 (<https://www.ibm.com/uk-en/analytics/spss-statistics-software>) and Microsoft Excel (www.microsoft.com). Second order polynomials were fitted to evaluate the relationship between agarose gel concentrations and both RBT and E values; Pearson's correlation analysis was used to assess correspondence between E values and RBT measurements. Two-way mixed repeated measures ANOVA was performed to test the difference in RBT measurements (8 scleral readings) as the within-subject variable and ethnic group (BW, BS and HKC) as the between-subject variable. Multiple three-way mixed repeated measures ANOVAs were performed to test the influence of ethnicity and between-subject factors relating to refractive status (i.e. with myopia *versus* without-myopia), axial length grouping³⁵ (1: $21.5 > - \leq 23.5$; 2: $23.5 > - \leq 25.5$) gender (males and females) and age (years)^{36,37} median split (1: $18 > - \leq 29$; 2: $29 > - \leq 40$) (Table 1). A stepwise forward multiple linear regression was used as an exploratory test to determine which

Table 1. Average Coefficient of Variance (CoV%) for rebound tonometry (RBT) on agarose gels of different concentrations

Agarose concentration (w/v%)	CoV (%)
0.25	5.27
0.50	2.81
0.75	6.68
1.00	7.72
1.25	4.32
1.50	6.87
1.75	6.51
2.00	7.36

biometric and demographic variables best explained the variation in scleral RBT values. Intra- and interobserver variability was calculated using intraclass correlation coefficient (ICC; two-way mixed single measures (consistency agreement); ICC (consistency, k (number of raters) = subject variability/ (subject variability + measurement error/ k)) in SPSS. Coefficients of variance (CoV% = [standard deviation/mean] * 100) were calculated for both the corneal and scleral intraobserver data as well as the agarose RBT readings for different concentrations. CoV data from individual agarose vials across the 8 different concentrations of agarose were further tested via a one-way ANOVA. For all statistical tests a *p*-value of <0.05 was taken as the criterion for statistical significance.

RESULTS

Calibration of the RBT against agarose gels

A non-linear relationship was observed between increasing concentration of agarose and the corresponding *E* and RBT values (Figure 1a). Notably, as the concentration of agarose increased the variability of the *E* values also rose (Figure 1a). The overlapping of the standard deviations in Figure 1a suggest that there is little difference between these two curves. The similarity in the \times square terms for both polynomials further confirms that the shape of the curves are alike. RBT readings showed a significant correlation with agarose *E* values ($r = 0.987$, $p < 0.001$) (Figure 1b).

Average repeatability of RBT readings on the 10 vials of each concentration of agarose gels showed the 0.50% RBT readings to provide the lowest CoV (2.81%) and 1% the highest (7.72%). Despite the increased variability in *E* and RBT values with higher concentrations of agarose (Figure 1b), repeatability does not show a commensurate decrease and remains below 8% for all concentrations. The CoV of the five RBT readings per vial showed significant differences between the different concentrations ($F_{7,71} = 5.91$, $p < 0.001$); Bonferroni *post hoc* test demonstrated statistically significant differences between the RBT readings for the 0.25% and 0.50% agarose gels and the 6 remaining concentrations.

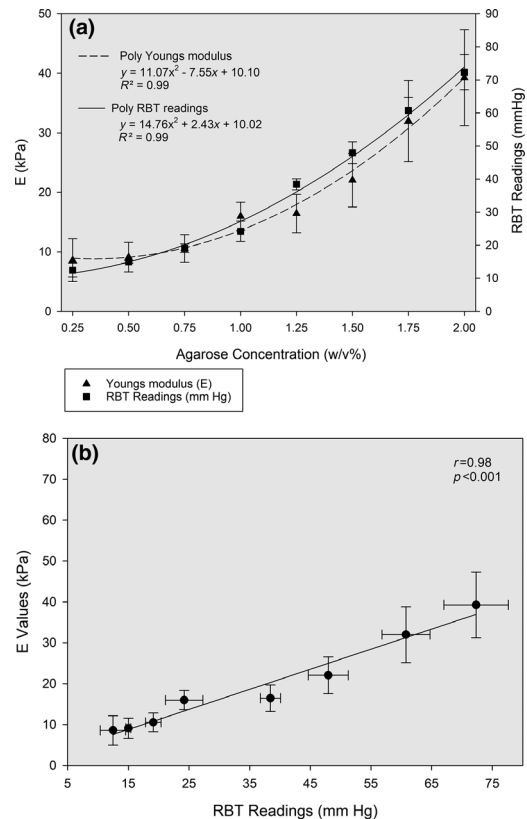


Figure 1. (a) Agarose concentration (%w/v) versus both mean *E* (kPa) and mean rebound tonometry (RBT) values (mmHg). (b) Mean RBT values (mmHg) versus mean agarose *E* values (kPa). Error bars \pm 1 S.D.

Application of the RBT tonometer to the sclera

Scleral RBT measurements were obtained on 130 individuals (Table 2).

Regional variation

A significant difference in scleral RBT readings was observed ($F_{5,25,661.63} = 127.78$ $p < 0.001$) with maximum mean RBT readings observed at IN and minimum at SN (Table 3); Bonferroni *post hoc* test revealed statistically significant differences between all regions except between SN: T, T:I, IT:I, IT:N, I:N.

Ethnicity

Ethnicity demonstrated a significant effect on the scleral RBT ($F_{2,124} = 14.38$ $p < 0.001$) (Figure 2); Games Howells *post hoc* analysis revealed that the scleral RBT readings for all regions amongst the HKC individuals was significantly higher than those for the BW ($p < 0.001$) and BSA ($p = 0.015$) groups. A significant ($F_{10,44,647.41} = 2.55$ $p = 0.004$) interaction effect was found between ethnicity and regional variation of the scleral RBT readings, which on examination of the interaction plot appeared to be

Table 2. Descriptive data of mean (1 S.D.) on the cohorts assessed (BW, British-White; BSA, British South-Asian and HKC, Hong Kong Chinese)

Ethnic groups	BW, n = 48	BSA, n = 22	HKC, n = 60
Gender (male:female)	16:32	11:11	31:29
Age (years)	28.8 (5.3)	24.8 (4.1)	25.0 (4.6)
Group 1 (18>–≤29)	n = 27 25.0 (3.0)	n = 20 23.9 (2.8)	n = 49 23.4 (3.2)
Group 2 (29>–≤40)	n = 21 33.7 (3.2)	n = 2 34.0 (4.2)	n = 11 32.2 (2.8)
Rx (DS)	–1.47 (3.04)	–2.80 (4.04)	–3.57 (3.09)
Without- myopia	n = 26 +0.52 (1.15)	n = 9 +0.49 (1.06)	n = 11 +0.39 (0.66)
With myopia	n = 22 –3.83 (2.89)	n = 13 –5.07 (3.76)	n = 49 –4.46 (2.70)
Axial length (mm)	24.02 (1.36)	24.67 (1.51)	25.35 (1.35)
Group 1 (21.5>–≤23.5)	n = 22 22.94 (0.61)	n = 8 23.09 (0.64)	n = 6 23.22 (0.25)
Group 2 (23.5>–≤25.5)	n = 19 24.27 (0.55)	n = 7 24.73 (0.52)	n = 28 24.69 (0.60)
Group 3 (>25.5)	n = 7 26.46 (0.80)	n = 7 26.42 (0.61)	n = 26 26.54 (0.93)

attributable to regions SN and S; notably, SN and S showed the largest difference in RBT values between the three ethnic groups.

Refractive status and axial length

No significant difference in scleral RBT values were observed between individuals with- and without-myopia ($F_{1,125} = 1.54$, $p = 0.22$) or between axial length grouping ($F_{2,124} = 1.24$, $p = 0.29$).

Gender and age

Gender ($F_{1,125} = 9.79$, $p = 0.002$) and age grouping ($F_{1,125} = 7.05$, $p < 0.009$) both showed a significant effect, with males and the older age group showing higher RBT values for all regions.

Influence of biometric and demographic factors on scleral RBT values

All potential confounding variables that were likely to affect scleral RBT values were evaluated in multiple regression models with the regional scleral RBT measurements as the dependent variables. As an exploratory test, all of the variables (i.e. ethnicity, axial length, refractive error, IOP, gender and age) were included in the initial model and the best predictors determined. Due to high levels of multicollinearity (i.e. >0.80) between refractive error and axial length multiple regression models were evaluated with each parameter included separately. Axial length and refractive error were not statistically significant in any of these multiple regression models (Table 4). With the exception of the inferior region, ethnicity was found to be the chief predictor for scleral RBT values for all regions. The level of variance accounted to ethnicity varied between the regions ranging from 38.1% superiorly to 7.3% inferior-temporally. Albeit having a lesser degree of effect, age was also found to be a significant predictor for all regional RBT values.

Intra- and inter-observer variation of RBT measurements

No significant examiner differences were found for the intraobserver RBT values; average ICC values were 0.98. Average CoV indicated the cornea to show the highest repeatability (4.04%) and the IN region the least (15.43%). Similarly, interobserver correlation coefficients also demonstrated highest repeatability for the cornea and the least repeatability for quadrants IT, I and N (Table 5).

DISCUSSION

The novel finding of increasing RBT values with agarose biogels of higher Young's modulus and the high levels of correlation between the two confirms its proposed utility for providing surrogate non-invasive clinical measures of material stiffness. Moreover, the overlapping standard

Table 3. RE corneal and anterior scleral rebound tonometry (RBT) ((mean (1S.D.) and range) mm Hg) readings for each ethnic group

Ethnic group	BW	BSA	HKC	Average
Location	RBT values (mean (S.D.)) and range			
Cornea	14.99 (3.22) (10.00–26.00)	15.40 (2.13) (11.00–19.33)	15.09 (3.58) (10.00–22.00)	15.10 (3.23) (10.00–26.00)
SN	29.55 (8.87) (15.75–54.00)	30.01 (9.76) (16.75–53.67)	42.19 (12.12) (24.00–75.33)	35.41 (12.25) (15.75–75.33)
S	25.10 (7.19) (14.50–40.00)	27.52 (9.66) (15.25–50.50)	39.56 (8.68) (22.75–58.67)	32.07 (10.76) (14.50–58.67)
ST	21.32 (7.31) (12.25–39.25)	23.73 (8.07) (14.50–40.50)	29.75 (8.21) (13.25–48.00)	25.59 (8.73) (12.25–48.00)
T	33.31 (11.16) (15.50–68.00)	34.34 (12.99) (13.50–61.50)	40.75 (10.85) (22.67–75.50)	36.92 (11.81) (13.50–75.50)
IT	38.23 (11.22) (21.60–69.75)	39.52 (11.82) (21.67–61.50)	45.34 (12.07) (7.00–67.25)	41.73 (12.11) (7.00–69.75)
I	36.98 (14.14) (9.33–70.00)	39.95 (15.90) (23.00–68.33)	43.21 (15.15) (17.00–73.50)	40.34 (15.07) (9.33–73.50)
IN	46.13 (12.53) (30.00–82.50)	49.325 (15.32) (27.25–84.50)	55.53 (14.00) (30.50–96.00)	51.01 (14.28) (27.25–96.00)
N	39.91 (10.29) (23.25–71.67)	41.695 (11.65) (24.25–63.25)	48.41 (12.17) (23.50–80.25)	44.13 (12.02) (23.25–80.25)

Regional scleral RBT readings were significantly different ($p < 0.001$) except between SN:T, T:I, IT:I, IT:N, I:N; Hong Kong Chinese (HKC) subjects showed significantly higher readings than the British-White (BW) ($p < 0.001$) and British South-Asian (BSA) ($p = 0.015$) groups.

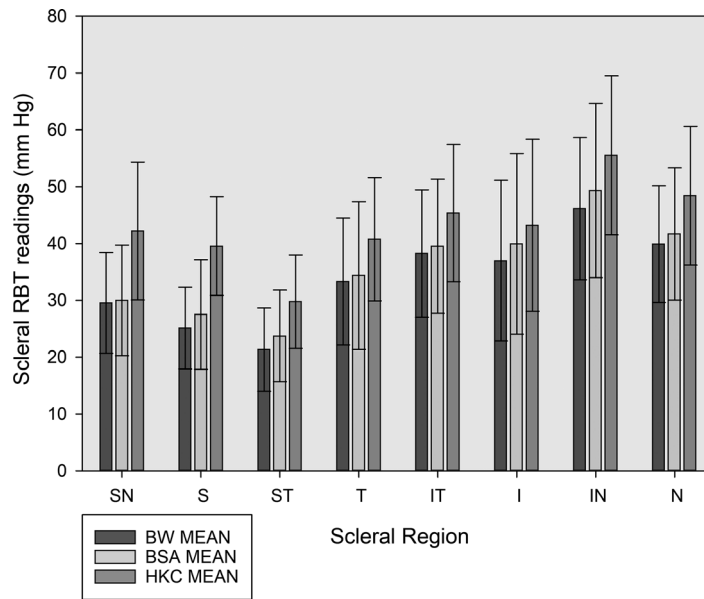


Figure 2. RE Mean \pm 1S.D. (error bars) scleral rebound tonometry (RBT) values at different regions of the anterior sclera for each ethnicity group. All regions amongst the Hong Kong Chinese (HKC) individuals were significantly higher than those for the British-White (BW) ($p < 0.001$) and British South-Asian (BSA) ($p = 0.015$) groups.

deviations and similarity in the shape of the curve when both RBT and Young's modulus were plotted against agarose gels of increasing concentration confirms that both metrics are measuring the same biomaterial property of the gels. The application of RBT for proxy measures of scleral resistance is unique; however, it has been previously trialed for the evaluation of corneal biomechanics.^{16,21,38,39} RBT measures of scleral resistance in mmHg do not provide measures of elastic modulus as reported in literature^{40–42} but instead offer scope to measure relative differences in regional scleral resistance.

Amongst all ethnic groups the superior-temporal region was found to show the least tissue resistance (low RBT readings); values sequentially increased across the inferior regions reaching maximum inferior nasally (high RBT readings). Albeit a slightly different sequence of change in mechanical resistance between the regions, the present observations mirror reports of regional variation in scleral resistance from our lab where only four scleral regions were assessed via indentation tonometry.⁹ RBT provides a composite measure of both IOP and tissue resistance^{37,39}; the relative regional differences in scleral resistance observed here are unlikely to be due to localized differences in IOP across the circumference of the anterior globe but more plausibly a consequence of variation in collagen infrastructure and geometric structure i.e. thickness, curvature and shape. While a direct relationship between *in vivo* measures of scleral thickness and resistance cannot be made the parallels in the sequence of thickness change from thickest inferior nasally to thinnest superiorly presents a persuasive

case for an association^{10,14,43}; a thicker sclera inferior nasally is likely to offer greater mechanical resistance. Meridional variation in scleral curvature^{11,12,44} as well as a non-uniform conformation of the anterior segment^{24,45,46} may also contribute to the heterogeneity across the scleral RBT readings.

Despite the findings of non-significant differences in scleral resistance between refractive status and axial length grouping, noteworthy differences in scleral resistance between ethnicities were identified. Higher RBT values for all scleral regions amongst the predominantly myopic HKC group suggest that differences in anterior segment biometry⁴⁷ and ocular topography⁴⁸ between the ethnicities may provide some explanation.⁴⁷ Racial differences in posterior scleral compliance have been noted^{22,23} but little is known of such effects on the anterior sclera. Interestingly, age was found to modulate the scleral RBT readings amongst all ethnic groups, with higher scleral resistance being observed in the older age group. Reduced scleral compliance coupled with increased mechanical stiffness^{22,23} and increased thickness¹³ with age has been reported and ascribed to increased enzymatic and non-enzymatic cross-links between collagen fibres.^{36,49,50} Gender differences in RBT readings were also identified; males demonstrated significantly greater scleral resistance than females and the greater anterior scleral thickness in males^{10,13} may partly explain these differences.

Inferred measures of scleral resistance may have clinical relevance. Recently, microneedles have been shown to be a highly localized and minimally invasive vehicle for drug delivery, however better knowledge of *in vivo* scleral

Table 4. Multiple linear regression parameter estimates of variables related to the regional scleral rebound tonometry (RBT) measurements

RBT					
location	Significant factor	b	SE b	β	R^2 change
SN	Constant	-11.83	7.33		
	Ethnicity	7.85	1.02	0.583***	0.23***
	Age	0.82	0.18	0.341***	0.10***
	Corneal IOP	0.61	0.28	0.159*	0.03*
	Total R^2				0.35
S	Constant	-3.47	6.20		
	Ethnicity	8.06	0.81	0.682***	0.38***
	Age	0.60	0.14	0.286***	0.07***
	Corneal IOP	0.51	0.26	0.151*	0.02*
	Gender	-3.08	1.40	-0.143*	0.02*
Total R^2				0.49	
ST	Constant	-4.37	5.47		
	Ethnicity	4.46	0.74	0.465***	0.20***
	Age	0.60	0.13	0.351***	0.10***
	Corneal IOP	0.59	0.19	0.218**	0.06***
	Gender	-3.53	1.25	-0.202**	0.03*
Total R^2				0.38	
T	Constant	7.64	6.43		
	Ethnicity	5.09	1.11	0.392***	0.09***
	Age	0.71	0.20	0.307***	0.08***
	Total R^2				0.17
IT	Constant	23.99	7.45		0.07
	Ethnicity	4.31	1.15	0.324***	0.07**
	Age	0.62	0.20	0.264**	0.06**
	Gender	-4.94	1.99	-0.204*	0.04*
Total R^2				0.18	
I	Constant	3.38	8.38		
	Age	1.00	0.26	0.343***	0.06**
	Ethnicity	5.02	1.45	0.303***	0.08***
	Total R^2				0.14
IN	Constant	19.63	8.39		
	Ethnicity	6.14	1.30	0.391***	0.09***
	Age	1.03	0.23	0.370**	0.12***
	Gender	-5.54	2.24	-0.193*	0.04*
Total R^2				0.25	
N	Constant	6.73	6.22		
	Ethnicity	6.04	1.08	0.457***	0.11***
	Age	0.94	0.19	0.401***	0.14***
	Total R^2				0.25

Independent variables: gender, age, ethnicity, refractive error, axial length and corneal IOP.

* $p < 0.05$;

** $p < 0.01$;

*** $p \leq 0.001$.

mechanical properties is warranted for improved designs.⁵¹ The viability of scleral collagen cross-linking has been confirmed in animal models of myopia with observations of increased scleral biomechanical strength coupled with a reduced rate of axial elongation.^{52,53} Safety concerns surrounding the invasive nature of the procedure^{54,55} and the

Table 5. RE Intraobserver Coefficient of Variance (CoV%) and average intraclass correlation (ICC) coefficient values for rebound tonometry (RBT) on the cornea and sclera

Location	Average CoV (%)	ICC average measure
Cornea	4.04	0.92
SN	8.30	0.87
S	9.42	0.86
ST	8.60	0.79
T	9.80	0.82
IT	10.29	0.60
I	9.14	0.30
IN	15.43	0.84
N	7.37	0.39

inability to assess *in vivo* biomechanical changes to the sclera have precluded the application of scleral CXL in human myopia.⁵⁶ Furthermore, nonselective adenosine receptor antagonist, 7-methylxanthine (7-MX) has been shown to increase scleral strength and slow axial growth although the site of action of the 7-MX is unknown.⁵⁷ It is unclear how scleral CXL would be administered in the human eye or whether 7-MX affects the anterior sclera but the possible means of assessing pre- and post-treatment changes in scleral mechanical strength with the RBT needs further exploration.

When considering the utility of RBT for measures of anterior scleral resistance, fundamental differences between the material and geometric features (e.g. stress-strain response, hydration, IOP, variation in thickness and shape of the sclera) of the *in vivo* human sclera and agarose gel need to be considered. Indeed, from a biomechanics perspective the nature of scleral resistance that underpins the degree of *in vivo* measures of RBT is extremely complex and in this regard the technique is limited. However, the present findings offer scope for investigating the potential for developing a validation system using biological materials such as agarose to allow a standardised procedure to quantify *in vivo* anterior scleral resistance.

The concordance between RBT and Young's modulus is clearly evident across the range assessed however the higher variability with increased concentrations of agarose gels is likely to be due to the more viscous nature of the higher concentration gels which are more prone to greater heterogeneity in the gel matrix and unevenness of the meniscus surface.⁵⁸ The assessment of the reliability and repeatability of applying RBT to the sclera revealed good levels of repeatability with average CoV under 10% for all regions except the inferior- nasal and -temporal aspects. The inter-observer results mirrored this trend with high repeatability in most areas apart from the nasal, inferior and inferior temporal regions. Although the iCare RBT manual specifies that the device can measure between 5–50 mmHg, several

studies have demonstrated its applicability for higher tonometric readings. Lower reliability of the RBT at high IOPs (23–60 mmHg) has been noted with results in the order of 65% being within ± 3 –5 mmHg of Goldmann tonometry (GAT) IOPs (Ruokonen *et al.*, 2006);^{59,60} with a general trend for systematically higher RBT readings with rising IOP.⁶¹ Increased variability at elevated IOP levels may partly explain the poorer reproducibility observed for both increasingly stiffer agarose gels and scleral regions prone to higher RBT values. Few investigators have examined the reliability beyond 60 mmHg but when applied to the sclera of living human eyes of IOPs of up to 80 mmHg, Kontiola (1997)⁶² reported a strong correlation ($r = 0.87$) between GAT results and both deceleration time and maximum, key metrics used by the RBT to derive IOP readings.¹⁵ Consistent average repeatability with CoV levels below 8% for the RBT readings from agarose gels of different concentration provided confidence in the utility of RBT for measures of the scleral RBT particularly at higher levels of IOP (>50 mmHg). Coupled with the present observations of a strong correlation between the agarose E values and the RBT values, it would suggest that the RBT can be used for higher IOP readings but caution needs to be exercised for values greater than 50 mmHg.

Other reasons for the regional difference in reproducibility may be attributable to variation in anatomy and surface properties between the different locations and suggests that caution should be exercised when assessing RBT readings from these regions of relatively high variability. Factors such as the proximity of the site of measurement to muscle insertion points and tendons may be of relevance, since the local biomechanics of the tissues and collagen arrangement are likely to be altered in those regions.^{32,63} Conjunctival changes, especially nasally and temporally where early pinguecula are commonly seen, may partly explain these observations, although in the age group assessed herein no obvious changes were noted. Although RBT is relatively tolerant to changes in probe-eye distances (i.e. within a range 3–5 mm) and angle of impact (up to 10–20 degrees from normal),^{15,64,65} several studies have reported that variation in impact points on the cornea may affect IOP readings.^{37,39,66} Possible discrepancies in the location of the scleral measurements during the inter- and intra-observer reliability study may have contributed to the variability found. Despite this inconsistency in repeatability across locations, the results indicate that RBT appears to be a viable method of assessing anterior scleral resistance.

CONCLUSION

Findings from the present study are the first to show regional variation in anterior scleral resistance using RBT. The present results indicate that measurements of scleral

resistance using RBT are sufficiently robust to assess anterior scleral resistance *in vivo*. These results would imply significant scope for the development of a validation system using biological materials such as agarose to allow a standardised procedure to quantify anterior scleral resistance.

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Conflict of interest

The authors report no conflicts of interest and have no proprietary interest in any of the materials mentioned in this article.

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