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Evaluation of the relationship of corneal biomechanical metrics with physical intraocular pressure and central corneal thickness in ex vivo rabbit eye globes

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ABSTRACT

The relationship of corneal biomechanical metrics provided by the Ocular Response Analyzer (ORA) and Corvis ST (CVS) with physical intraocular pressure (IOPp) and central corneal thickness (CCT) was evaluated. Thirty fresh enucleated eyes of 30 rabbits were used in ex vivo whole globe inflation experiments. IOPp was measured with a pressure transducer and increased from 7.5 to 37.5 mmHg in steps of 7.5 mmHg while biomechanical data was acquired using the ORA and CVS. At least 3 examinations were performed at each pressure level, where CCT and twelve biomechanical metrics were recorded and analyzed as a function of IOPp. The biomechanical metrics included corneal hysteresis (CH) and corneal resistance factor (CRF), obtained by the ORA. They also included the applanation times (A1T, A2T), lengths (A1L, A2L) and velocities (A1V, A2V), in addition to the highest concavity time (HCT), peak distance (PD), radius (HR) and deformation amplitude (DA), obtained by the CVS. The variation of CCT and the twelve biomechanical metrics for the 30 rabbit eyes tested across the 5 pressure stages considered (inter-pressure differences) were statistically significant ($P = 0.00$). IOPp was highly to moderately correlated with most biomechanical metrics, especially CRF, A1T, A1V, A2V, PD and DA, while the relationships with CH, A2T, A1L and HCT were poor. IOP has important influences on most corneal biomechanical metrics provided by CVS and ORA. Two biomechanical metrics A1V and HR were influenced by CCT after correcting for the effect of IOP in most pressure stages, while the correlation with others were weak. Comparisons of research groups based on ORA and CVS with different IOPs and CCTs may lead to possible misinterpretations if both or one of which are not considered in the analysis.

1. Introduction

The cornea accounts for more than two thirds of the refractive power of the eye and is an important part of the outer, mechanically-tough, ocular tunic, which protects the eye's internal components (Fatt, 1978). Because of its importance to clear vision, a great deal of research has been conducted to understand its performance and how it responds to disease and surgery (Jue and Maurice, 1986), (Kurita et al., 2007). This included careful evaluation of the health and adequacy of the cornea to identify keratitis, increase the accuracy of corneal refractive surgery and prevent iatrogenic keratectasia (Seiler et al., 1998). In addition, the cornea's mechanical properties, essential for maintaining its dimensional stability and hence clear vision, rely primarily on the cornea's thickness and the biomechanical behavior of the tissue (Liu and Roberts, 2005), this topic has attracted increasing attention in recent years (Ali et al., 2014; Mikula et al., 2014; Wolffsohn et al., 2012).

Understanding the cornea's biomechanical behavior is important for several clinical applications. A clear example is refractive

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surgery where refractive error is corrected by controlled removal of stromal tissue, leading to alteration of corneal curvature. The weakness caused by tissue removal and in some cases separation of tissue layers, in addition to the changes in tissue behavior caused by wound healing, all have an important effect on the surgical outcome and should be understood to improve the predictability of surgical procedures (Roberts, 2000), (Roberts, 2005). The cornea's biomechanical properties have also been shown to have a significant effect on the accuracy of intraocular pressure (IOP) measurements (Wernreb et al., 2007), (Hamilton and Pye, 2008), and this effect becomes compounded in eyes that have undergone ablative refractive surgery (Liu and Roberts, 2005). The assessment of biomechanics is important for other applications which include the stiffness deterioration associated with keratoconus and the subsequent cross linking treatment or use of corneal implants, the mechanical interaction between the cornea and contact lenses, limbal incisions to correct astigmatism, and non-ablative refractive procedures such as conductive keratoplasty (CK).

Several methods and instruments (Bonatti et al., 2009; Luce, 2005; Scarcelli et al., 2012; Wang et al., 1996) have been developed to investigate the biomechanical response of the human cornea in vivo; ORA and CVS are two representative, more recent, non-contact devices that have been introduced in 2005 (Luce, 2005) and 2010 (Ambrósio et al., 2011a, 2011b), respectively. Clinically measured metrics provided by ORA and CVS have been widely used to assess the biomechanical response of the cornea (Leung et al., 2013; Pinero et al., 2010), with all of the metrics relying on the morphologic response of cornea to the action of the instruments' air puff indentation. Current evidence points at effects of IOP and CCT on some of ORA and CVS mechanical metrics (Chang et al., 2010; Kotecha et al., 2006), and while it is expected that the metrics will be related to the cornea's and possibly the sclera's biomechanical behavior, the link between these metrics and the established mechanical properties of tissue (such as tangent modulus and Poison's ratio) has not been established yet.

In this study, we seek to investigate the relationship between the biomechanical metrics produced by the ORA and CVS on one hand, and physical IOP and CCT on the other. In order to enable use of physical (true) IOP, the study relies on ex-vivo rabbit eyes rather than human eyes, in which it will be difficult to control IOP in vivo or obtain ex vivo specimens in sufficient numbers.

2. Materials and methods

2.1. Specimen preparation

Thirty Japanese big ear white purebred rabbits (2–3 kg) from the Animal Breeding Unit at Wenzhou Medical University were included in this study. All animals were treated in agreement with the ARVO Statement for Use of Animals in Ophthalmic and Vision Research and with the approval of the Animal Care and Ethics Committee of the Eye Hospital, Wenzhou Medical University. The rabbits had their intraocular pressure (IOP) measured using a Tono-pen tonometer (Reichert, Inc., New York, USA) to ensure the eyes were not subjected to elevated IOP since the long-term exposure to high IOP could potentially influence the corneal biomechanical properties (Sun et al., 2009). They were euthanized by intravenous injection of high concentration of pentobarbital sodium (Merck, Darmstadt, Germany). One of the bilateral eyes were randomly chosen, immediately enucleated while keeping the iris intact in each day. Each experiment was completed within 4 h of enucleation at room temperature. The whole research lasted for 30 days.

2.2. Corneal biomechanical metrics measurement

An infusion needle, connected with a transfusion bottle and a pressure transducer, was inserted into the vitreous body of the eye through the optic nerve to control the IOP (Fig. 1). The transfusion bottle, filled with Phosphate Buffered Saline (PBS, Maixin, China), was hung on a movable tank that was raised and lowered to change the pressure in the eye. This pressure was continuously monitored by the pressure transducer and the measurements assumed to equal the physical IOP (IOPp). The pressure was set at five specific levels (7.5, 15, 22.5, 30, 37.5 mmHg) and at each level measurements by two air puff devices; the Ocular Response Analyzer (ORA, Reichert, Inc., New York, USA) and the Corvis ST (CVS, Oculus Optikgeräte GmbH, Wetzlar, Germany), were made. The pressure step of 7.5 mmHg was adopted for experimental convenience, being equal to 1 kPa, and the range of pressure considered was thought adequate for covering the range seen in ophthalmic practice from normal to high IOP. Sufficient time (<2 min in all cases) was allowed for the pressure transducer readings to stabilize before commencing with ORA and CVS measurements. CCT was measured by the CVS at each pressure level. Three ORA and CVS measurements were taken at each pressure level and the average values used for statistical analysis. A period of at least two minutes was left between each two measurements at each manometric level in order to avoid the stress history of each measurement event affect subsequent readings.

Cornea was found to be slightly oedematous after using the Corvis,so measurements with the Corvis were always conducted first then followed by the ORA. The ORA provided two biomechanical metrics: the corneal hysteresis (CH); the difference between the 2 applanation pressures (P_1 and P_2); and the corneal resistance factor (CRF), which is designed to have a strong correlation with CCT. Both CH and CRF are reported to be influenced by the cornea's viscoelastic behavior (Roberts, 2014). The dynamic Scheimpflug imaging analysis of CVS provides ten parameters related to corneal deformation under the effect of air pressure. These are the first and second applanation times (A1T and A2T), length of the flattened cornea at the first and second applanations (A1L and A2L), maximum corneal velocity at the two applanation events (A1V and A2V), time from air-puff starting until the point of highest concavity of the cornea (HCT), radius of curvature of corneal concavity at the time of the highest deformation (HR), distance between the two peaks of the cornea at the time of the highest concavity (PD) and the maximum deformation amplitude of the cornea at the highest concavity (DA).

2.3. Statistical analysis

All analyses were performed using the PASW Statistics 20.0 (SPSS Inc., Chicago, USA). Comparison of these parameters was performed using MANOVA of repeated measurements. The result of MANOVA of repeated measurements was expressed as F (df1,df2), where df1 = degrees of freedom 1 and df2 = degrees of freedom 2. In this study, P-values of less than 0.05 were considered to be statistically significant. A second-order polynomial regression was used to explore the nonlinear correlation between IOPp and the twelve biomechanical metrics. The correlation of CCT and twelve parameters was assessed by means of the Pearson's or Spearman linear correlation factor according to normal distribution test in bilateral corneas.

3. Results

Table 1 shows the intraclass correlation coefficient (ICC) values for the three consecutive biomechanical response measurements