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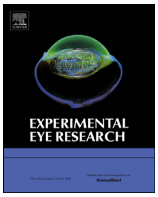

Refractive index degeneration in older lenses: A potential functional correlate to structural changes that underlie cataract formation

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ABSTRACT

A major structure/function relationship in the eye lens is that between the constituent proteins, the crystallins and the optical property of refractive index. Structural breakdown that leads to cataract has been investigated in a number of studies; the concomitant changes in the optics, namely increases in light attenuation have also been well documented. Specific changes in the refractive index gradient that cause such attenuation, however, are not well studied because previous methods of measuring refractive index require transparent samples. The X-ray Talbot interferometric method using synchrotron radiation allows for measurement of fine changes in refractive index through lenses with opacities. The findings of this study on older human lenses show disruptions to the refractive index gradient and in the refractive index contours. These disruptions are linked to location in the lens and occur in polar regions, along or close to the equatorial plane or in lamellar-like formations. The disruptions that are seen in the polar regions manifest branching formations that alter with progression through the lens with some similarity to lens sutures. This study shows how the refractive index gradient, which is needed to maintain image quality of the eye, may be disturbed and that this can occur in a number of distinct ways. These findings offer insight into functional changes to a major optical parameter in older lenses. Further studies are needed to elicit how these may be related to structural degenerations reported in the literature.

1. Introduction

The power of a lens to refract light is the prime measure of its functional capacity. In the case of the biological lens, its refractive power, which depends on the curvature and refractive index is linked to the distribution and concentration of its constituent proteins (Pierscionek and Augusteyn, 1991, 1992; Keenan et al., 2008, 2009; Grey and Schey, 2008; Slingsby et al., 1997 Slingsby, 1985; Pierscionek and Regini, 2012). The functional capacity of the lens to refract is contingent on relatively unimpeded passage of light through its medium. When cataract develops and disrupts the transmission of light through the lens, the structure/function relationship between the proteins and the optical properties is altered. Histological and microscopic studies that have looked at fine structure in lenses with cataract, or in older lenses that show changes similar to those found in cataractous lenses, indicate the major structural degradations that underlie the process of opacification (Costello et al., 1992, 2008; Gilliland et al., 2004; Al-Ghoul and Costello, 1993, 1996; Al-Ghoul et al., 1996; Metlapally et al., 2008; Costello et al., 2012; Brown et al., 1989, 1993; Vrensen and Willekens, 1990; Bassnett et al., 2011). Conclusions have been drawn about how structure maintains transparency and hence what structural degradations may affect loss of function (Bassnett et al., 2011) but these structural studies have not been complemented with investigations of functional, optical parameters to the same level of detail.

Whilst it is known that the two major attenuation factors that manifest clinically as cataract, are scatter and absorption of light, there is a paucity of studies on the optics of intact lenses and the changes in the refractive index that may indicate early stages of opacification. This is because methods of measuring refractive index require or assume that light will be refracted and not scattered or absorbed (Pierscionek and Regini, 2012). One study that used

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fibre optic sensing to measure refractive index from the amount of reflected light in very localised regions of the lens, found significant attenuation in the reflections from central regions of older lenses and concluded that these most likely indicated optical changes caused by structural degradation (Pierscionek, 1997).

To measure with accuracy how refractive index changes in older and cataractous lenses requires a method that can detect localised refractive index fluctuations without affecting measurements in regions of the lens that can still transmit light. The recent development of an interferometric method using a synchrotron X-ray source has made it possible to measure refractive index directly and accurately in any given plane of whole lenses within the eyeball (Hoshino et al., 2011). This method can detect fluctuations in refractive index that show structural degradation and differentiate these from normal fluctuations in the refractive index gradient (Hoshino et al., 2011; Bahrami et al., 2014) because it does not require the sort of assumptions necessary in other methods that have been used to determine or estimate refractive index in intact lenses (reviewed in Pierscionek and Regini, 2012).

This study on refractive index variations in older donor lenses has found functional characteristics that show forms of deviation from the refractive index gradient beyond the physiological fluctuations found in healthy younger samples (Bahrami et al., 2014). The deviations indicate abnormalities in the refractive index gradient that may be relevant to structural degradations and provide the basis for defining and understanding early cataractous changes in the optical parameters of the lens.

2. Methods

Older human eyes (15 samples aged from 72 to 88), from which corneal discs were removed for transplantation, were obtained from the Bristol Eye Bank (UK) and transported in dry ice to the SPring-8 synchrotron radiation facility in Japan where they were placed at −20 °C. Samples were moved in pairs from −20 °C to +4 °C at 12–13 h before measurement and placed at room temperature around 3 h before measurement. The same procedure was applied to all samples in order to maintain similar pre-experimental conditions. Thawed lenses were removed from eyeballs and set in an agarose gel that was physiologically balanced, within a specially designed sample holder. Samples were set in pairs, one above the other. Refractive index variations was measured using the X-ray Talbot grating interferometer (Momose, 2005; Momose et al., 2003) as described previously (Hoshino et al. (2011); Bahrami et al., 2014). The interferometer uses a 25 keV X-ray beam at the bending magnet beamline BL20B2. X-rays pass through a Si(111) double crystal monochromator and two transmission gratings. The first was a tantalum phase grating (G1) with pattern thicknesses 2.1 μm and the second a gold absorption grating (G2) (pattern thickness of 16.6 μm). Both gratings have a grating pitch of 10 μm and a pattern size area of 25 mm. Moire fringes are detected by the beam monitor and a scientific CMOS detector (ORCA Flash 4.0. Hamamatsu Photonics). A Piezo stage was used to shift G2 for phase retrieval and a 5-step ‘on-the-fly’ fringe-scan method was used. Phase shifts were calibrated against solutions of known density and experimental results compared to phase shifts per pixel which were converted to refractive index as described in Hoshino et al. (2011). The measurement on each sample took 50 min providing 900 scans for each lens. Repeat measurements were conducted on two lenses. The error in the measurement of refractive index was ±0.0005.

Refractive index values in three-dimensional spatial coordinates were processed using Mathematica computational software v9 to plot iso-indicial contours for increments of 0.002 in refractive index. The study was approved by the National Health Service (NHS) ethics committee (Oxford, UK).

3. Results

Fig. 1A to E shows refractive index contours in sagittal planes of five lenses. Changes in the colour density represent changes in refractive index with each adjacent contour indicating an incremental step changes of 0.002 in refractive index. In four of these figures, notably Fig. 1A to D, the refractive index contours are distorted or show localised areas of relatively large density fluctuations that represent disturbances in the refractive index profiles. These disturbances are categorised into four types: those that occur in the region of the optic axis (three lenses with representative shown in Fig. 1A), those that are located close to or within the equatorial plane (five lenses with representative shown in Fig. 1B), those that follow directions that are oblique to the optic axis and the equatorial plane and are close to the equatorial region (four lenses with representative shown in Fig. 1C) and those that appear as denser layers: ie disturbances that run along contours that approximate the outer shape of the lens (three lenses with representative shown in Fig. 1D). In four lenses more than one type of disturbance was found. This is seen in Fig. 1D that shows evidence of disturbances which appear to follow lamellae as well as slight density changes in the polar region. The lamellar-type patterns are also seen with density fluctuations in the equatorial plane or in planes oblique to the optic axis. There is no evidence, within those lenses that have disturbances in the polar regions, of any fluctuations that run parallel or within the equatorial plane. Fig. 1E shows the refractive index contours for an 86 year old lens with no evidence of significant disturbances to the index contours indicating that these changes are not found in all older lenses and are therefore most likely to be a manifestation of changes that may be indicative of early opacification and underlying cataractous processes rather than ageing.

The refractive index profiles in the sagittal plane for the lenses given in Fig. 1 are shown in Fig. 2. The fluctuations in the nucleus are seen in Fig. 2A–D which correspond to samples where perturbations in the refractive index contours are seen. The changes are most dramatic for the profile in Fig. 2A which shows a sharp cleft in the refractive index corresponding to the density disturbances seen in Fig. 1A. The refractive index dips to around 1.412 with maxima of 1.426 and 1.435 seen on either side. In Fig. 2B and C where the refractive index disturbances are, respectively, parallel to the equatorial plane and in orthogonal directions, the refractive index profiles have sharper drops and the profiles are distorted. Fig. 2D, which corresponds to the refractive index profile with disturbances that run along contours, has no fluctuations in refractive index in the sagittal plane that are beyond those seen in younger lenses (Bahrami et al., 2014). Instead, consistent with lamellar density changes (Fig. 1D), there is a sharp definition between the almost flat portion of the refractive index profile and the cortical regions that show a steep gradient with fluctuations at the intersections. A slight distortion of the profile is also evident with a steeper cortical gradient in the posterior than in the anterior part of the lens. The refractive index profile in Fig. 2E is relatively smooth and symmetrical, akin to that seen in younger lenses (Bahrami et al., 2014) with some small fluctuations in the central, nuclear part of the profile.

The corresponding refractive index contours in the equatorial plane for the same set of lenses are given in Fig. 3. Fig. 3A, which corresponds to disturbances in refractive index in the polar regions, has very few fluctuations but a slight elevation in refractive index at around 2 mm on either side of the centre of the profile (marked with arrows) and a sharp dip in the centre of the profile (marked with an arrow). Where disturbances occur within the equatorial