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Analysis of nuclear fiber cell cytoplasmic texture in advanced cataractous lenses from Indian subjects using Debye–Bueche theory

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Abstract

Alterations in ultrastructural features of the lens fiber cells lead to scattering and opacity typical of cataracts. The organelle-free cytoplasm of the lens nuclear fiber cell is one such component that contains vital information about the packing and organization of crystallins critical to lens transparency. The current work has extended analysis of the cytoplasmic texture to transparent and advanced cataractous lenses from India and related the extent of texturing to the nuclear scattering observed using the Debye–Bueche theory for inhomogeneous materials. Advanced age-related nuclear cataracts (age-range 38–75 years) and transparent lenses (age-range 48–78 years) were obtained following extracapsular cataract removal or from the eye bank, at the L.V. Prasad Eye Institute. Lens nuclei were Vibratome-sectioned, fixed and prepared for transmission electron microscopy using established techniques. Electron micrographs of the unstained thin sections of the cytoplasm were acquired at 6500× and percent scattering for wavelengths 400–700 nm was calculated using the Debye–Bueche theory. Electron micrographs from comparable areas in an oxidative-damage sensitive (OXYS) rat model and normal rat lenses preserved from an earlier study were used, as they have extremely textured and smooth cytoplasms, respectively. The Debye–Bueche theoretical approach produces plots that vary smoothly with wavelength and are sensitive to spatial fluctuations in density. The central lens fiber cells from advanced cataractous lenses from India and the OXYS rat, representing opaque lens nuclei, produced the greatest texture and scattering. The transparent human lenses from India had a smoother texture and less predicted scattering, similar to early cataracts from previous studies. The normal rat lens had a homogeneous cytoplasm and little scattering. The data indicate that this method allowed easy comparison of small variations in cytoplasmic texture and robustly detected differences between transparent and advanced cataractous human lenses. This may relate directly to the proportion of opacification contributed by the packing of crystallins. The percent scattering calculated using this method may thus be used to generate a range of curves with which to compare and quantify the relative contribution of the packing of crystallins to the loss of transparency and scattering observed.

Keywords: aged; lens nucleus; cataract; cytoplasm; humans; scattering; electron microscopy; Fourier analysis

1. Introduction

The ocular lens is designed to be transparent and transmits visible light to the retina with negligible absorption and scattering. Transparency of the ocular lens depends primarily on its ultrastructure. The lack of cellular organelles along the light path (Bassnett, 2002), close packing of fiber cells to minimize extracellular space (Taylor et al., 1996), organization of its predominant component proteins (crystallins) (Bettelheim and Siew, 1983; Delaye and Tardieu, 1983) and the conspicuous absence of large fluctuations in refractive index contribute to this crucial feature of the lens (Benedek, 1971; Bettelheim, 1985; Zigler, 1994; Kuszak and Costello, 2004). Cataract, defined as any opacity of the lens, is characterized by overall

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diminished contrast at the retina due to both absorption and increased scattering of incident light leading to visual deficit (Duncan, 1981; Brown and Bron, 1996; Datiles and Magno, 2004; Costello and Kuszak, 2008). Age-related nuclear cataracts, the most common form of human cataracts (Murthy et al., 2007), begin as white hazy scattering in the nuclear core and progressively involve more of the nucleus as the cataract matures. Ultrastructural features that have been reported from our laboratory to be contributory to the scattering and opacity of human lens nuclei include enlarged extracellular spaces, extracellular space deposits (Costello et al., 1992; Al-Ghoul et al., 1996), distinct 1–4 μm globular particles called multilamellar bodies (Gilliland et al., 2001, 2004) and texturing of fiber cell cytoplasm seen in thin sections (Al-Ghoul and Costello, 1996; Taylor and Costello, 1999; Freel et al., 2002).

An important observation from these ultrastructural studies was that early or immature age-related nuclear cataracts were similar in morphology to transparent non-cataractous lenses (Al-Ghoul and Costello, 1996; Al-Ghoul et al., 1996; Kuszak et al., 1998; Freel et al., 2002), except for a greater number of large scattering particles, the multilamellar bodies (Gilliland et al., 2001, 2004). However, few such detailed comparisons have been possible with advanced cataracts due to methodological reasons, especially problems of availability and adequate fixation of the interior of the dense ocular lens. The study of advanced age-related nuclear cataracts may provide valuable data regarding the physical basis of transparency and the mechanisms of cataract formation, and may corroborate or invalidate widely held theories about these aspects of lens research. Moreover, this may also provide us with clues for delaying the onset of cataract or addressing the critical need to devise preventative strategies for cataract, which is the leading cause of blindness in the world, particularly in developing countries (Thylefors et al., 1995; Dandona et al., 2001; Foster, 2001; Nirmalan et al., 2003).

The current study has concentrated specifically on the ultrastructure of cytoplasmic texturing seen in dehydrated and embedded thin-section micrographs of nuclear tissue from advanced age-related cataracts. This feature is directly related to the packing, density and organization of crystallins within lens fiber cells. Biochemical studies have reported that crystallins aggregate into high-molecular-weight particles (Jedziniak et al., 1973; Spector and Roy, 1978; Zigler, 1994; Benedek, 1997) and this is generally accepted to be the underlying cause of scattering seen in nuclear cataracts. The size of these presumably distinct particles, in theory, should be in the range 20–200 nm to produce significant scattering (Benedek, 1971; Bettelheim, 1985; Clark, 1994), a size range that can be visualized easily in thin-section electron micrographs. Yet, high-molecular-weight aggregate particles have not been observed within the cytoplasm of well-preserved human nuclear fiber cells (Costello et al., 1992; Al-Ghoul and Costello, 1996; Al-Ghoul et al., 1996; Taylor and Costello, 1999; Freel et al., 2002). Fourier analysis has been used to quantify cytoplasmic texturing in age-related nuclear cataracts (possibly from protein modification or redistribution) and to relate it to the scattering (Taylor et al., 1997; Taylor and Costello, 1999; Freel et al., 2002; Costello and Johnsen, 2004), as the technique is sensitive to small variations not perceived by eye. Results from these studies show that the cytoplasm in mild age-related cataracts is similar to age-matched transparent lenses and in these instances cytoplasmic variations do not account entirely for the observed scattering.

An extension of detailed ultrastructural analyses to more advanced age-related nuclear cataracts extracted during surgery from blind subjects in India has offered preliminary information regarding the innermost fiber cells (Costello et al., 2006). In the current study, we have estimated the contribution of the packing and organization of the fiber cell cytoplasm to light scattering from the in vivo lens nucleus in transparent lenses to lenses with a range of opacities. The well-established Debye–Bueche theory for inhomogeneous materials (Debye and Bueche, 1949) has been used in this analysis, as has been used in the past for characterizing scattering from the lens (Bettelheim and Paunovic, 1979; Bettelheim et al., 1981; Bettelheim and Siew, 1983), because this theory is robust and directly applicable to thin sections of densely packed amorphous proteins. The analysis indicates that the organization of the cytoplasm is altered markedly in advanced age-related nuclear cataracts and is a major contributor to the total light scattering by cataractous nuclei.

2. Materials and methods

2.1. Lens material

Lens nuclei from advanced age-related nuclear cataracts (graded 3–4 on a 0–4 scale by clinicians; $n = 10$) were obtained from subjects in the age-range 38–75 years, who underwent extracapsular cataract extractions at L.V. Prasad Eye Institute, Hyderabad, India. The nuclei were brought from the operating room to a laboratory in the facility in a vial containing gauze moistened with saline at room temperature immediately following extraction. Patient information obtained was limited to the age, gender, diabetes mellitus status and the nuclear cataract grading by the surgeon, following guidelines of the Institutional Review Board for protection of human subjects. Transparent whole donor lenses ($n = 4$; age-range 48–78) obtained from the Ramayamma International Eye Bank located within the Institute had limited information available, usually only the age and gender. All lenses were obtained according to the tenets of the Declaration of Helsinki. Oxidation sensitive (OXYS) ($n = 1$) and normal rat ($n = 1$) lenses preserved from earlier studies (Marsili et al., 2004) were used for comparison.

2.2. Fixation and sectioning

Lenses obtained were examined using a hand-held slit lamp (model 510, Eidolon Corporation, MA, USA) and had their equatorial diameters and thicknesses measured using calipers. They were sectioned into 200 μm thick slices using a Vibratome (series 1000, St. Louis, MO, USA). The freshly cut slices were then prepared as described in earlier studies (Freel et al., 2002). In brief, they were immersed in modified Karnovsky's fixative