In Vitro Generation of Functional Lens-Like Structures with Relevance to Age-Related Nuclear Cataract

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PURPOSE. To investigate the capacity of lens epithelial cells, maintained in a modified explant culture system, to mimic normal patterns of lens cell differentiation and to regenerate lens structure and function.

METHODS. Lens epithelial explants were set up in pairs with their apical surfaces facing each other. These explant pairs (EPs) were then cultured in vitro for up to 43 days to promote their growth and differentiation. Immunohistochemistry and conventional light and electron microscopy were used to assess structural and functional properties of the lens-like structures that developed from EPs.

RESULTS. EPs that were asymmetrically exposed to vitreous routinely produced biconvex, lens-like structures composed of ordered epithelial and fiber cells that were transparent and had some focusing and magnifying ability. In addition, characteristics of the lens in vivo, like fiberlike cells that were peripherally situated in EPs contained markers of the relatively early stages of fiber differentiation, whereas centrally situated cells contained markers of terminally differentiated fibers. During long-term culture of the EPs, a central opacity appeared that had structural features similar to those reported for the early stages of human, age-related nuclear cataract.

CONCLUSIONS. This study shows that, given appropriate culture conditions, lens epithelial cells can regenerate ordered lens-like structures with functional properties. This system represents a valuable new tool for the investigation of factors involved in the generation of normal lens structure and function and lens opacification. (Invest Ophthalmol Vis Sci. 2007;48: 1245–1252) DOI:10.1167/iovs.06-0949

Cataract is the most common cause of blindness in the world today. Estimates from the World Health Organization indicate that of the 45 million people blind worldwide, approximately 20 million are blind because of cataract. Relatively little is known about the precise mechanisms that underlie cataract formation and development. As a result, the most effective treatment for cataract is surgery, which involves removal of the cataractous material and insertion of an intraocular lens. Although this procedure is generally effective in restoring sight, a common complication is the development of posterior capsule opacification (PCO) that arises from abnormal growth and differentiation of lens epithelial cells left behind after surgery. These cells proliferate and migrate along the exposed capsule surface. Some form plaques of fibroblastic/myofibroblastic cells that cause lens capsule wrinkling and eventually interfere with the passage of light along the visual axis. When these complications arise, further treatment is often given to try to restore some visual acuity; however, this is not without complications, and it adds further costs to what is the most common surgery carried out in developed countries.

Given the predicted worldwide increase in the incidence of cataract, there is a clear need to inhibit or delay cataract formation and to minimize or prevent PCO. Much research has been aimed at devising ways of reducing the incidence of PCO by eliminating all epithelial cells from the capsular bag, but these approaches have not yet proved to be effective and have the potential to induce other serious complications. An alternative approach is to devise strategies that provide detailed knowledge of the processes involved in the development of normal lens structure and function. With this information it may be possible to identify novel ways of delaying the development of cataract and promoting the normal growth and differentiation of lens cells to regenerate features of normal lens structure and function after cataract surgery. Evidence that this knowledge-based approach is feasible comes from studies with lens epithelial explants, which have shown that the addition of vitreous, or growth factors such as FGF, can promote aspects of lens cell differentiation. To date, these explant experiments have mainly been directed at studying the molecular processes involved in cell differentiation and identifying factors that promote these processes; few attempts have been made using in vitro systems to specifically regenerate organized structures with lens-like functional properties, such as transparency and focusing ability. In the present study, we modeled some aspects of embryonic lens development in vitro to generate structural and functional features of the normal lens. By manipulating a lens epithelial explant system, we produced lens-like structures composed of ordered epithelial and fiber cells that were transparent and had some focusing ability. Prolonged culture of these lens-like structures also showed features that resembled those described in human cataracts. These results indicate that this system could be a useful in vitro model for identifying factors involved in the generation and degeneration of lens structural and functional properties.

MATERIALS AND METHODS

Collection of Ocular Fluids

Bovine eyes were placed on ice immediately after harvesting and were transported to the laboratory. Ocular fluids were collected within approximately 6 hours of the eyes harvest, as previously described. Briefly, aqueous fluid was collected with a sterile syringe and a 22-gauge needle inserted into the anterior chamber through the central cornea, after which the anterior portion of the eye was removed with...
described. With the use of a dissecting microscope and jewelers were removed and placed in medium (M)199 that had been equili-
mittee. All experimental procedures with animals conformed to the ARVO
Statement for the Use of Animals in Ophthalmic and Vision Research
Preparation and Culture of Explant Pairs (EPs)
ion was maintained. The first explant was then returned to the incubator for approximately 15 minutes. Epithe-
cell explants were subsequently collected from the paired lenses with jewelers forceps to gently tear the lens capsule adjacent to the
ner suture and slowly remove the lens fiber mass. The first explant collected was pinned to the culture dish such that the capsule was in contact with the culture dish and the cells were uppermost. A second epithelial monolayer was placed on top of the first monolayer such that the two monolayers overlapped with their cells in direct contact. Equatorial lens epithelial cells were then trimmed from both monolayers and the resultant EPs cultured in vitreous-containing medium.

the lens attached. Vitreous fluid from 20 eyes was collected and pooled using a sterile syringe without a needle. The pooled vitreous was homogenized with a syringe and a 19-gauge needle, portioned into aliquots, and stored at –20°C for up to 4 weeks.

**Preparation and Culture of Explant Pairs (EPs)**

All experimental procedures with animals conformed to the ARVO
Postnatal day (P)21 Wistar rats were killed, and then their eyes were removed and placed in medium (M)199 that had been equili-
ated in a tissue culture incubator set at 37°C in 5% CO2, as previously described. With the use of a dissecting microscope and jewelers forceps, the eyes were immediately torn open at the optic nerve to release the lens. Randomly paired lenses were placed into 35 x 10-mm culture dishes containing equilibrated M199, after which the dishes were returned to the incubator for approximately 15 minutes. Epithelial cell explants were subsequently collected from the paired lenses with jewelers forceps to gently tear the lens capsule adjacent to the posterior suture and slowly remove the lens fiber mass. The first explant collected in was in contact with the culture dish and the cells were bathed by the culture medium (Fig. 1). The second explant was then placed on top of the first so that the two epithelial layers were in contact and overlapped across their entire areas (Fig. 1). The peripheral regions of both explants were trimmed away to ensure that any cells that might have been stimulated to differentiate in vivo were not present during the culture period. Explant-pairs (EPs) thus generated were cultured in a 1:1 mixture of M199 and bovine vitreous, with the culture medium/vitreous changed every fifth day of culture.

**Analysis of Focusing Ability**

The ability to focus light was defined during this study as the ability to increase and then decrease the amount of white light detected within a constant area grid-square over a fixed distance (Fig. 2). The size of the grid-square used was determined empirically for each EP but followed the general rule of (d/3 x d/3), where d was the side length of the tissue measured (Fig. 2A). The fixed distance was 4.8 mm, the range of movement of the objective lenses on an inverted microscope (DMIL, Leica, West Ryde, Australia).

Culture dishes were placed on the stage of the inverted micro-
scope, and an EP was brought into focus with the 20× lens (Fig. 2B). This position was denoted the 0° position. With the use of a digital camera (DC100, version 2.51; Leica), a live image of the cultured EP was obtained. The stage was then moved, without changing focus, so that only the culture medium, not the EP, was in the light path. Using the brightness level (BL) function of the digital camera software, an indicator of the amount of light reaching the camera detector, the amount of light coming from the microscope lamp was adjusted so that the BL detected by the camera was 250 units (saturation of the digital camera detector occurred at approximately 480 units). After this cali-
the BL recorded after each incremental movement (Fig. 2B). Once the maximum travel distance (4.8 mm) had been reached, the objective lens was returned to the 0° position, the stage was moved so that only the culture medium was in the light path, and the BL measurements were repeated on the culture medium alone using the same grid-square size. To analyze the data, the BL measurements were graphed (Fig. 2C). For each EP, the BL recorded at each incremental position was plotted (Fig. 2C). For the culture medium, average BL measurements were generated for each incremental position by averaging all the culture medium BL values recorded at a particular incremental position. These average BL values were then plotted with their SD (Fig. 2C).

**Analysis of Magnifying Ability**

The culture dish containing the EP to be analyzed was placed on a dissecting microscope directly above a printed x, thereby ensuring that the distance between the EP and the x was constant between culture dishes. The EP was brought into focus with a 25× ocular lens, and a photograph was taken of the x underneath medium alone or under-
neath each individual EP with a digital camera (DC100; Leica) and accompanying software (version 2.51). From these images the central width of the x under medium alone or under each individual EP was measured. To determine whether the cultured EPs were capable of magnifying the x, the ratio between the central width of the x under medium alone to the average central width underneath the EPs was calculated. Data were analyzed by the one sample t test with the use of a statistics program (GraphPad Prism, version 2.0; GraphPad Software Inc., San Diego, CA).

**Confocal Microscopy**

The cellular arrangement of differentiating cells within EPs was ana-
yzed under a confocal microscope using the membrane stain DiOC6 (3,3’-dihexyloxacarbocyanine iodide; Molecular Probes, Eugene, OR) and the nuclear stain EtBr (ethidium bromide; Sigma, St. Louis, MO). EPs were fixed for 20 minutes with 10% neutral-buffered formalin (NBF), washed in phosphate-buffered saline (PBS), and stained overnight at 4°C with 5 µg/mL DiOC6. Stained EPs were washed repeatedly at room temperature with PBS before they were mounted in 10% PBS over glycerol. Images of stained EPs were obtained with a scanning system (Confocal Scanning System; Bio-Rad, Hercules, CA) and accompanying software (LaserSharp2000; Bio-Rad). The 488-nm laser was used to visualize DiOC6.
Electron Microscopy

The morphology of differentiating fiber cells within EPs was analyzed with transmission electron microscopy (TEM). EPs were fixed with 3% glutaraldehyde, postfixed with 1% osmium tetroxide, and embedded for TEM, as previously described. TEM images were obtained using an electron microscope (JEM-1010; JEOL, Brooksvale, NSW, Australia) and collected using a digital camera and accompanying software (Bio-Scan; Gatan, Pleasanton, NJ).

Immunofluorescence

EPs were analyzed by immunofluorescence for the distribution of various lens cell proteins. All primary antibodies were used at 5 μg/mL according to previously described protocols. Primary antibodies against α-, β-, and γ-crystallin were prepared as previously described, and commercial antibodies were purchased for vimentin (Dako, Botany, NSW, Australia) and protein disulfide isomerase (Stressgen, Victoria, BC, Canada).

RESULTS

Focusing Ability of Cultured Explant Pairs

To assess the feasibility of generating a functional lens-like structure in vitro, EPs from P21 rats were asymmetrically exposed to vitreous fluid to simulate aspects of normal lens development (Fig. 1). In these conditions, the EPs took on a progressively ovoid shape and, after approximately 30 days, were transparent and appeared to be able to focus light (Fig. 2D). Measurements of the amount of light at various distances below the central region of the cultured EPs confirmed that they had developed focusing capability (Fig. 2E).

Magnifying Ability of Cultured EPs

Because of its biconvex shape and unique, crystallin-based refractive index, the ocular lens can magnify images. Because cultured EPs were able to focus light, they were also analyzed for their ability to magnify a printed x (Fig. 3A). Image taken under a dissecting microscope showed that by 37 days of culture, the tissues were able to magnify images (Fig. 3B). The magnification factor in Figure 3B was approximately 1.8, whereas the average magnification factor for all the EPs in that experiment was approximately 1.6 (Fig. 3C). Data analysis from this experiment using the one sample t test revealed the difference between the sizes of the magnified and unmagnified central regions of the printed x to be statistically significant (P = 0.0017).

Interestingly, continued culture of the EPs resulted in loss of lens function. Images taken after 43 days of culture showed that EPs commonly developed opacities and that this resulted in reduced focusing ability. This was observed when cultured...
EPs were placed above a printed x (Fig. 3D) and when the light intensity data for these opacified EPs was plotted on a graph (Fig. 3E).

Cellular and Molecular Composition of Cultured EPs

Because lens protein composition and distribution of specific lens cell types is considered one of the factors responsible for lens function,18 immunofluorescence was used to investigate the distribution of characteristic lens proteins in EPs toward the end of the culture period. EPs were analyzed for the presence of five proteins expressed in the lens: α-, β-, and γ-crystallin, the cytoskeletal protein vimentin, and the endoplasmic reticulum protein called protein disulfide isomerase (PDI). These five proteins were chosen because their expression patterns in the lens are indicative of different stages in lens fiber differentiation. For example, lens epithelial cells express α-crystallin, vimentin, and PDI but undetectable amounts of β- or γ-crystallin. In contrast, cells in the early stages of lens fiber differentiation begin to accumulate β-crystallin and then, after a time, γ-crystallin while continuing to express α-crystallin, vimentin, and PDI. As the fiber cells progress through the later stages of differentiation, restructuring of the developing fiber cell cytoskeleton and loss of the fiber cell organelles results in terminally differentiated lens fibers that contain α-, β-, and γ-crystallin, but not vimentin or PDI.17,19–24

Analysis of the EPs at day 40 of culture demonstrated that these tissues had many lens-like features. They were ovoid and had monolayered epithelial-like cells at their peripheries (Figs. 4A, 4B). These cells expressed α-crystallin, vimentin, and PDI but not β- or γ-crystallins (Figs. 4C–G, insets). Although these epithelial-like cells were consistently found in the region of the explant not directly exposed to the culture medium (facing the bottom of the culture dish), variable-sized groups of these epithelial-like cells were also found in the vitreous-exposed region of the tissues. Further examination of the remainder of the EPs revealed two readily distinguishable populations of fiberlike cells. A large population of cells at the center of the P21-derived EPs contained α-, β-, and γ-crystallin but little, if any, vimentin or PDI (Figs. 4C–G). This indicated that the cells in this central region were in the relatively late stages of lens fiber differentiation. In contrast, a smaller population of fiberlike cells contained vimentin and PDI, as well as α- and β-crystallin, but did not contain γ-crystallin (Fig. 4C–G, asterisks). This indicated that these cells were at an earlier stage of lens fiber differentiation than the cells in the central region of the tissue. Although the size of this population of younger fiberlike cells varied between individual EPs, this population was consistently found in the peripheral region of the tissue that was directly exposed to the culture medium.

Nuclear Morphologies within Cultured EPs

Examination of the cultured EPs indicated the presence of two populations of fiberlike cells that differed in their degree of differentiation; therefore, cultured tissues were analyzed for nuclear morphologies characteristic of different stages of lens fiber differentiation. Analysis of the tissues by transmission electron microscopy (TEM) showed that the epithelial-like cells located at the peripheries of the EPs were cuboidal and contained large nuclei and abundant organelles, similar to lens epithelial cells seen in vivo (Fig. 5A).12,25–27 The fiberlike cells at the peripheries of the EPs had rod-shaped nuclei that were darker staining than the surrounding cytoplasm and had prominent nucleoli (Fig. 5B). In contrast, the fiberlike cells in the center of the EPs contained nuclei with distinctly different appearance. Nuclei in these cells were smaller, circular, had a fragmented nuclear envelope, were similarly stained compared...
with the surrounding cytoplasm, and contained fragmented nucleoli (Fig. 5C). Other fiberlike cells within the center of the cultured P21 tissues contained what appeared to be a condensed nucleolarlike substance, unassociated with any other visible nuclear structures (Fig. 5D).

Cellular Arrangement within Cultured EPs

Confocal microscopy showed that most cells in EPs were long, thin, and parallel aligned (Fig. 6A). Individual elongated cells were several hundred microns in length. Further analysis of the tissues by TEM showed that the elongated fiberlike cells were closely packed with limited extracellular space (Fig. 6B) and had hexagonal profiles when sectioned perpendicularly to

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**FIGURE 4.** Cellular and molecular composition of EPs. Immunofluorescence on midsagittal sections of 40-day cultured EPs. (A) The low-power Hoechst-stained EP shows the lens-like ovoid shape of the tissue. Epithelial-like cells were adjacent to the surrounding lens capsule, consistently forming a continuous monolayer on the lower side of the tissue (the side that faced the base of the culture dish; arrows). The boxed region is represented at higher magnification in the serial sections. (B-G) Dotted line: surrounding lens capsule. The region indicated by the arrows in (A) is represented at higher magnification in the insets in (B-G). Immunofluorescent localization is shown for Hoechst (B), α-crystallin (C), β-crystallin (D), γ-crystallin (E), vimentin (F), and PDI (G). These data indicate a large mass of cells located within the center of cultured EPs, containing molecular markers characteristic of mature fiber cells. (C-G) Presence of α, β, and γ-crystallins but no vimentin or PDI. In contrast, cells at the EP periphery often contained characteristic markers of immature fiber cells (yellow asterisks indicate the presence of α- and β-crystallins, vimentin, and PDI; no γ-crystallin was present). Scale bars: (A) 80 μm; (B-G) 20 μm; insets, 10 μm.

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**FIGURE 5.** Nuclear morphologies in different regions of EPs. (A) An epithelial-like cell from a region of the EP that was in contact with the culture dish. These cells are adjacent to the lens capsule (Ca) and typically contain a large, lobulated nucleus (asterisks) and numerous organelles. (B) A rod-shaped nucleus from an elongated cell in the peripheral region of the EP. Nuclei in these cells were darker staining than the surrounding cytoplasm and contained prominent nucleoli. (C) A nucleus from a centrally located fiberlike cell. These nuclei routinely had circular profiles, indistinguishable nuclear substance and cytoplasm, and degenerating nuclear membranes. (D) Other centrally located cells contained small, condensed, nucleolarlike material not associated with any other nuclear structure. Scale bars: (A, B) 1 μm; (C) 1.5 μm; (D) 0.85 μm.

Ultrastructure of Opaque EPs

In addition to the features described, TEM revealed the presence of other structures in the EPs around the time the tissues became cloudy (approximately day 43; see Fig. 5). EPs that became opaque were shown by TEM to contain membrane whorls encircling regions of cytoplasm of different densities (Fig. 7A). Groups of small globules that did not appear to be contained within multiple membranes were sometimes present (Fig. 7B). Numerous structures with circular profiles (Figs. 7C, 7F) were often, but not always, found near the cell membrane. These structures exhibited variable staining compared with the surrounding fiberlike cells and could be found alone or in groups of varying sizes. Because these circular structures were composed of multiple membranes, they were termed multilamellarlike bodies (MLLBs) after the multilamellar bodies (MLBs) first identified by Gilliland et al.20 Closer examination of these MLLBs revealed variations in their degrees of membrane separation. Within some of the MLLBs, the membranes were separated by approximately 8 to 10 nm of cytoplasmic material (Figs. 7D, 7E), whereas in others the membranes appeared to be separated by little, if any, cytoplasmic material (Figs. 7F-L), suggesting progressive compaction of the space separating the membranes during their formation. The membranes found in the MLLBs were approximately 7 to 8 nm
thick and in this regard were similar to the more typical plasma membranes of the fiberlike cells (data not shown).

DISCUSSION

Development of Lens Function and Structure

To generate in vitro lens tissues with normal structural and functional features, we modified the decades-old rat lens explant culture system. During lens morphogenesis, cells of the lens vesicle face each other; anterior cells differentiate into the lens epithelium, and the posterior cells, exposed to vitreous humor, differentiate into fibers. To mimic aspects of this cellular arrangement, we set up paired epithelial explants that were asymmetrically exposed to vitreous. Our results show that when cultured for more than 30 days, the EPs produced an approximately biconvex tissue capable of focusing light and magnifying images. Functional EPs contained epithelial-like and fiberlike cells that had structural, ultrastructural, and molecular features similar to those seen in the normal lens. For instance, by approximately 40 days of culture, EPs generally contained monolayers of cuboidal, epithelial-like cells that were primarily located in the region of the tissue that had not been in direct contact with the vitreous fluid (the part of the EP that faced the plastic surface of the culture dish). These cells had large,
lobulated nuclei similar to those seen in lens epithelial cells in vivo.\textsuperscript{75–27} Furthermore, these cells expressed proteins normally present in lens epithelial cells. They included α-crystallin, the cytoskeletal protein vimentin, and the endoplasmic reticulum protein PDI.\textsuperscript{17,19–24} In some cases, however, the epithelial cells extended toward the vitreous-exposed surface and, on rare occasions, were multilayered and encircled the tissue. Why the epithelial cells varied in location in this way is unknown. One possibility may be related to the different batches of vitreous used during this study. Although every effort was made to standardize vitreous collection, some variations between vitreous batches were unavoidable. For example, the ages of the animals from which the vitreous was collected varied from batch to batch and could therefore have introduced various nuclear and/or differentiation factor concentrations between experiments. Note also that the vitreous was routinely diluted 1:1 with culture medium, so that concentrations of such factors would already have been reduced by 50% at the outset of these experiments.

**Terminal Differentiation**

The cells that made up the bulk of the EPs were generally long, thin, parallel aligned, and contained ultrastructural and molecular markers of differentiated lens fiber cells. In vivo, in addition to the marked elongation and alignment of differentiating cells, lens fiber differentiation is characterized by the abundant expression of β- and then γ-crystallin,\textsuperscript{17,23} the development of complex membrane interdigitations,\textsuperscript{26,27,52} and the loss of organelles, including the nucleus.\textsuperscript{20,21,25–27,31} Detailed molecular analyses of the processes surrounding organelle loss and nucleolus during the normal lens have demonstrated that organelle loss can be monitored by the disappearance of the endoplasmic reticulum protein PDI.\textsuperscript{19–24} That nucleolus are precursors of the cytoskeletal protein vimentin.\textsuperscript{22,23} Similarly detailed analyses of nuclear morphologies within the lens have demonstrated a distinct progression of nuclear changes that occur during terminal differentiation.\textsuperscript{20,21,25–27,32} For instance, lens epithelial cells have large lobulated nuclei. As differentiation begins, the nuclei become rod shaped and stain darker than the surrounding cytoplasm, and distinct nucleoli appear. Later, as the cells begin to denucleate, the nuclei become circular and stain similarly to the surrounding cytoplasm, and the nucleoli condense to form spokelike configurations. As denucleation is completed, the nucleoli and nuclear envelope fragment, and the nucleus disappears. Finally, fragmented and condensed nuclear remnants are visible for some time in the terminally differentiated fiber cells.

Based on these molecular and ultrastructural changes, the fiberlike cells within the EPs could have divided into two distinct populations. One was a smaller, variably-sized population of fiberlike cells that contained markers of the relatively early stages of lens fiber differentiation and was located in the peripheral region of the tissue directly exposed to the vitreous-based culture medium. These cells contained α- and β-crystallins, but not γ-crystallin. Furthermore, these peripheral fiberlike cells contained vimentin and PDI, indicating that they had not undergone the specific cytoskeletal changes that precede denucleation nor had their organelles been degraded. The presence of organelles was further evidenced by darkly-staining, rod-shaped nuclei containing prominent nucleoli. The other was a larger, centrally-located population of fiberlike cells that contained markers indicating that these cells were undergoing, or had achieved, terminal lens fiber differentiation. These cells contained α-, β-, and γ-crystallins but did not contain vimentin or PDI, indicating that the cells had undergone specific cytoskeletal changes that precede denucleation and had begun to degrade their organelles. Further evidence that these cells had started, if not achieved, terminal differentiation was demonstrated by the nuclear morphologies within them. These morphologies included nuclei that were circular and similarly stained to the surrounding cytoplasm, with highly condensed nucleoli and fragmented nuclear envelopes. Furthermore, condensed nucleolar material could be seen that was not associated with any other nuclear structure. The presence of ball-and-socket interdigitations between many cells in this region also indicated that advanced differentiation had been achieved.

When viewed together, this arrangement of fiberlike cells in the relatively early stages of differentiation, outside a much larger population of more terminally differentiating fiberlike cells, parallels the arrangement of lens fibers found in vivo. In this regard, the system of using EPs provides a significant advance over the previous system of culturing single explants. Although studies in our laboratory showed that the culture of single epithelial explants from postnatal rats with vitreous or FGF could reproduce many structural features of fiber differentiation, there was no evidence of comparable replication of normal lens growth and maturation patterns, as seen in the present study.\textsuperscript{9,19} It is unclear how these advanced characteristics are achieved; however, a major difference from the previous culture system is that in an EP all the cells are contained within lens capsula (Fig. 1), and this substratum may provide more appropriate conditions for ordered growth, differentiation, and maturation of fiber cells. For instance, the presence of an epithelial sheet on one side and a capsule on the other side reproduces the in vivo arrangement where the anterior tips of differentiating/maturing fibers migrate along the epithelium and the posterior tips migrate along the posterior capsule. Understanding how the EPs develop ordered growth patterns is an important area for future studies because it may provide new information that could be applied to help direct more normal patterns of growth and differentiation of lens epithelial cells after cataract surgery.

**Features of Age-Related Cataract**

Aside from the development of features characteristic of normal lens structure and function, another significant finding from the present study was the loss of function that occurred with the long-term culture of the EPs. This was associated with the development of cloudiness within the center of the EPs, similar to that reported for the early stages of age-related nuclear (ARN) cataract in humans.\textsuperscript{53–35} Ultrastructural analysis of the opacified P21-derived EPs showed that, unlike shorter-term transparent cultured EPs, they contained membrane whorls and various structures with circular profiles. Interestingly, similar structures have also been reported to occur in ARN cataract.\textsuperscript{36,37} For example, Gilliland et al\textsuperscript{37} reported the presence of MLBs in human lenses with ARN cataract. These reported MLBs were approximately 1 to 3 μm in diameter, had circular profiles, exhibited variable staining compared with the fiber cell cytoplasm, contained 3 to 8 layers of closely packed, thin membranes (5 nm), and were consistent with mathematical predictions for size and abundance of irregularities required to produce ARN cataract. Although the multilamellar bodies (MLBs) within the opacified EPs had slightly thicker membranes (7–8 nm) and were sometimes contained more than eight closely packed layers, they were nevertheless similar to human MLBs in size, staining, and general appearance. In addition, MLBs were found in the cultured EPs with different degrees of membrane separation, suggesting a progressive compaction in the space separating the membranes during their formation. Given the similarities (and differences) that exist between the human MLBs and EP-derived MLBs, the latter may represent early stages of MLB formation.
in the maturing fiber cells. Understanding the molecular processes that result in the formation of MLLBs and the associated opacification in EPs could provide valuable information on the formation of ARN cataract. Notably, this in vitro system avoids difficulties associated with obtaining human cataractous lenses suitable for identifying and studying factors involved in cataract initiation and progression and therefore warrants further investigation.

Thus, taken together with the evidence that, given appropriate culture conditions lens epithelial cells can be induced to grow and differentiate in such a way as to reproduce important structural and functional properties of the ocular lens in vitro, this system represents a valuable new tool for the investigation of factors involved in both the generation and degeneration of structural and functional features of the mammalian lens.

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References


