Polygonal Arrays of Actin Filaments in Human Lens Epithelial Cells

An Aging Study

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In order to determine the importance of lens actin filament configuration to lens accommodation, the pattern of actin filaments in the epithelium was studied in human lenses from different decades of life spanning the accommodative and non-accommodative years. Polygonal arrays of microfilaments were demonstrated in whole mounts of epithelium from normal and cataractous lenses using rhodamine phalloidin, an actin-specific, fluorescent-labeled probe. Tangential section transmission electron microscopy (TEM) studies confirmed that these arrays consist of central vertices and interconnecting filament rays, which line the apical end of each epithelial cell and appear to attach to the lateral membrane. These polygonal arrays were present in human lenses ranging from 25–94 yr of age. Measurements of intervertex distance showed remarkable constancy throughout the ages studied. In view of these findings, it is proposed that a possible function of these polygonal arrays is to stabilize the lens epithelium during lens flattening. Invest Ophthalmol Vis Sci 27:1535-1540, 1986

Polygonal arrays of microfilaments have been described in a variety of cell cultures, including primary cultures of chick embryo cells and human lung fibroblasts, re-attaching early passage rat embryo cells, permanent cell lines, such as gerbil fibroma, non-neoplastic rat and hamster, rabbit epitheloid, and mouse 3T3. In these cultured cells, the polygonal network appears transiently before the termination of spreading and organization of stress fiber bundles. Cultured bovine lens epithelial cells also develop polygonal arrays and stress fibers. The arrays appear for a short time before the cells differentiate and then elongate into lens fibers. The functions of these networks are unknown, and arrays have yet to be demonstrated in the intact bovine lens. Tangential TEM sections of the intact rabbit lens and whole mounts of the lens epithelium have revealed microfilaments in arrays lining the inner apical plasma membrane of anterior epithelial cells. These microfilaments have been shown to contain actin using rhodamine phalloidin conjugate, a fluorescent-labeled, actin-specific probe. The rabbit lens, which has up to one diopter of accommodation, sometimes shows a greatly different "sequestered actin bundles" (SAB) pattern. The question, therefore, arises about the importance of lens actin structure in accommodation. One approach to answering this question is to study the pattern in human lenses from different decades in life spanning the accommodative and non-accommodative years. The purpose of this report is to describe the pattern of actin filaments in the epithelial cells of the in vivo human lens from 7 decades of life, as demonstrated by tangential-section TEM and by actin-specific rhodamine phalloidin staining.

Materials and Methods

Three groups of human lenses were used in this study: 1) cataractous lens epithelium removed via extracapsular cataract extraction; 2) cataractous lenses removed via intracapsular cataract extraction; and 3) postmortem normal lenses removed from eyes donated by the Illinois Eye Bank. The cataractous lenses were described as aging cataracts by the attending ophthalmologists, and required removal in order to restore useful vision. Use of this material was given approval by the University Human Subjects Review Committee.

Extracapsular Cataract Extraction

The eyes of 22 patients, aged 51–94 yr, were presurgically dilated by one of two methods: 1) Cyclogyl 1% (Alcon, Ft. Worth, TX) and Neosynephrine 2½% (Winthrop-Breon Labs, Des Plaines, IL), one drop of...
each every 10 min, two or three times; or 2) Murocoll 2 (Muro, Tewksbury, MA) one drop every 5 min, four or five times. The patients had been premedicated with Valium 5–10 mg orally, and surgery was performed after local retrobulbar anesthesia.

The anterior chamber was opened and an anterior capsulotomy done with the tip of the irrigating cystitome of the Kelman/Cavitron (Van Nuys, CA) phacoemulsifier unit. In two cases, the 51- and 94-yr-old, sodium hyaluronate (Healon; Pharmacia, Piscataway, NJ) was instilled into the anterior chamber before lens extraction. The anterior epithelium was removed with surgical forceps and transferred with a drop of balanced salt solution (BSS; Alcon, Ft. Worth, TX) to a microscope slide coated with 0.1% polylysine and spread to lie flat. The whole mount preparation was then allowed to dry, and was fixed within 1 hr after removal. Fixation was done in 3.7% formaldehyde in 0.12 M sodium phosphate buffer, pH 7.2 for 10 min at room temperature, followed by a wash in phosphate buffered saline (PBS) for another 10 min. The preparation was then permeabilized in absolute acetone at −20°C for 5 min. The preparation was air-dried and stored at 4°C until phalloidin staining. Since the orientation of the preparation could not be determined until after staining, only those which by chance had been placed with the capsule next to the slide were usable; 8 of 22 lenses were suitable for study.

Eye Bank Normal Lenses

Three pairs of normal human lenses from males aged 25, 30, and 50 yr were obtained, respectively, at 17, 3½, and 18 hr postmortem from the Illinois Eye Bank. The causes of death were multiple trauma, head trauma, and brain tumor, respectively. The eyes had been stored at 4°C in moist chambers. After the lenses were removed, anterior capsulotomies were done with forceps and scissors. A microscope slide was gently pressed onto the anterior capsule, and both capsule and epithelium were pulled off and spread to flatten the tissue. The whole mount preparations were treated as were those obtained by extracapsular cataract extraction, described above.

Phalloidin Staining

Rhodamine phalloidin (Molecular Probes Inc, Eugene, OR) was aliquoted in a small test tube and evaporated with a stream of gaseous nitrogen. The phalloidin was resuspended in 10 volumes of PBS, then layered over the lens preparation. The preparation was incubated for 20 min in the dark at room temperature, washed thoroughly in a stream of PBS, and a coverslip was mounted with Gelvatol (Elvanol; Monsanto, Springfield, MA).

Whole mounts were viewed with a Leitz Dialux-20 fluorescence microscope using a 540 nm filter and 63× or 100× Fluotar objectives. Photography was done with a Leitz Vario-Orthomat (Wetzlar, Germany) camera and Tri-X film exposed at ASA 1600. The film was developed in Diafine (Kodak, Rochester, NY).

Intracapsular Cataract Extraction and TEM

Five cataract patients, aged 72–85 yr, underwent intracapsular cataract extraction surgery. Their eyes were dilated either with Murocoll 2 or Cyclogyl 1%, as described above. They all had received oral Valium 5 mg before local retrobulbar anesthesia. The anterior chamber was opened, and 0.5 cc of 1:10,000 solution of α-chymotrypsin (Zolyse; Alcon, Ft. Worth, TX) was instilled. After 2 min, the anterior chamber was flushed with sterile BSS, and the lens was removed with a cryoprobe. The lens was gently thawed from the cryoprobe directly into a fixative.

Three lenses were fixed in 2% glutaraldehyde in 0.05 M sodium phosphate buffer, pH 7.2, containing 0.2% tannic acid and 0.002% CaCl2 for 30 min at room temperature. After having been rinsed in 0.5 M sodium phosphate buffer, pH 7.2, the lens was postfixed in 1% OsO4 in the same buffer, for 1 hr on ice. The lens was processed for TEM after dehydration through ethanol and propylene oxide, cut into sagittal slices, and embedded in Araldite 502 Resin (Electron Microscopy Sciences, Ft. Washington, PA) in orientations for tangential and cross sectioning.

Two lenses were fixed in 2% glutaraldehyde in 0.05 M sodium phosphate buffer, pH 7.2, for 30 min at room temperature. The lenses were dehydrated in ethanol and then placed into LR White (The London Resin Co., Ltd, Hampshire, England) medium with two changes over 3 days at 4°C. The lenses were cut, as above, and then embedded in LR White without accelerator at 50°C for 24 hr.

Araldite and LR White sections were cut with a Sorvall MT2B ultramicrotome. One micron thick sections were stained with Richardson’s methylene blue/azure II and used to determine apical ends of the epithelial cells in tangential sections. Seventy to eighty nanometer thin sections were cut and stained with 3% uranyl acetate in acetate buffer for 20 min, and then Reynolds’ lead citrate for 5 min. The sections were viewed with a JEOL (Tokyo, Japan) 100CX electron microscope operating at 60 kV.

Data Collection and Analysis of Intertvertex Distance

On enlarged photomicrographs of phalloidin stained cells, the centers (vertices) of the polygonal arrays were marked with a pinhole to increase the accuracy of their positions. An ink-tipped electronic stylus of a Sum-
Fig. 1. Fluorescence photomicrographs of human lens epithelial whole mounts after staining with the actin-specific probe, rhodamine phalloidin. Polygonal arrays containing actin filaments consist of central vertices appearing as white dots interconnected by rays of white-appearing filaments. a, 30-yr-old eye bank lens epithelium; b-f, epithelia from extracapsular cataract extraction; b, 51 yr; c, 66 yr; d, 75 yr; e, 85 yr; f, 94 yr. (×6,375)

magraphics Bit Pad One (Fairfield, CT) was used to record the distance between two adjacent pinholes (vertices). The data were recorded on a LSI-11 computer using a planimeter program. The data were analyzed with a statistics and a t-test program to determine whether the mean distances between vertices in the different aged lenses were significantly different.

In the TEM study, the distances between vertices were measured directly from the EM negatives.

Results

This study demonstrated that polygonal arrays of microfilaments can be seen in the anterior epithelium of all the human lenses investigated using a variety of techniques. Polygonal arrays were present in normal and in cataractous lenses, in lenses from 7 decades of life, in lenses fixed by two different electron microscopy techniques, and in whole mount preparations stained for fluorescence light microscopy.

The pictures obtained with the rhodamine phalloidin stain showed that the entire apex of each epithelial cell is lined with a blanket of central vertices with interconnecting rays (Fig. 1a-f). This staining pattern confirms that the arrays are, at least in part, made up of actin filaments.

A striking feature of these polygonal arrays is the constancy in the distance between the vertices.
Table 1. Intervertex distance determined from rhodamine-phalloidin fluorescence micrographs

<table>
<thead>
<tr>
<th>Lens age (Yr)</th>
<th>No. Measurements</th>
<th>Calculated Distance (µm) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>138</td>
<td>1.47 ± 0.36</td>
</tr>
<tr>
<td>30</td>
<td>167</td>
<td>1.51 ± 0.30</td>
</tr>
<tr>
<td>51</td>
<td>124</td>
<td>1.46 ± 0.28</td>
</tr>
<tr>
<td>66</td>
<td>207</td>
<td>1.46 ± 0.30</td>
</tr>
<tr>
<td>75</td>
<td>170</td>
<td>1.32 ± 0.28</td>
</tr>
<tr>
<td>85</td>
<td>200</td>
<td>1.35 ± 0.33</td>
</tr>
<tr>
<td>94</td>
<td>125</td>
<td>1.76 ± 0.46</td>
</tr>
</tbody>
</table>

Throughout the range of ages studied. Except for the 94-yr-old lens, with phalloidin fluorescence microscopy, the intervertex distance was between 1.31 and 1.51 µm. For the 94-yr-old lens, the mean distance was somewhat greater, 1.76 µm, but this value was not significantly different from the values for the younger lenses (Table 1). The polygonal arrays were somewhat disordered in the eye bank lenses in which a long delay between death and fixation ensued (Fig. 1a), and in the 94-yr-old lens, the numbers of arrays were reduced (Fig. 1f).

In the five whole cataractous lenses cut tangentially for TEM, polygonal arrays were seen to cover the apical epithelial membrane (Fig. 2). The number of rays of filaments varied between three and eight, depending in part on the angle of the cut, and some rays of filaments appeared to insert into the lateral plasma membrane (Fig. 3). The vertices were positioned fairly uniformly; intervertex distance averaged about 1.2 µm. Thus, the distance was nearly constant over the 6-yr age span between 72 and 79 yr (Table 2). The intervertex distance was slightly smaller in the preparations...
fixed for electron microscopy compared with those fixed for fluorescence microscopy. This discrepancy is thought to result from greater shrinkage induced by the EM protocols (prolonged dehydration, heat). The individual filaments of the rays measured 5–7 nm in diameter, which lends support to the actin specificity of the rhodamine phalloidin.

**Discussion**

From the fact that polygonal arrays were seen in non-cataractous eye bank lenses which had been stored up to 18 hr post-mortem, several factors may be inferred about these arrays. The existence of the arrays in the normal, young, and middle-aged lens demonstrates that these are not merely artifacts of cataract formation, of aging, or of the presurgical drug regimen. Also, these polygonal arrays demonstrate considerable stability against lysis, as the structure appears to be fairly intact even up to 17–18 hr post-mortem. The eye bank lenses whose fixation was delayed after death, —-

<table>
<thead>
<tr>
<th>Lens Age (Yr)</th>
<th>No. Measurements</th>
<th>Microscope Magnification</th>
<th>Calculated Distance (µm) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>72*</td>
<td>8</td>
<td>8,000</td>
<td>1.10 ± 0.12</td>
</tr>
<tr>
<td>73†</td>
<td>18</td>
<td>5,000</td>
<td>1.21 ± 0.19</td>
</tr>
<tr>
<td>73*</td>
<td>16</td>
<td>8,000</td>
<td>1.34 ± 0.13</td>
</tr>
<tr>
<td>79*</td>
<td>8</td>
<td>8,000</td>
<td>1.27 ± 0.18</td>
</tr>
</tbody>
</table>

* Lenses embedded in LR white.
† Lens embedded in Araldite (same lens photographed at different microscope magnifications).
however, showed fewer and less distinct rays of filaments between disordered vertices. Even in the less ordered network, the intervertex distances remained normal with the rhodamine-phalloidin stain.

The question arises about the function of these polygonal arrays and their possible association with accommodation. Polygonal arrays have been observed in the lenses of rabbit, squirrel, and monkey (Rafferty, unpublished data), all of which are capable of at least one diopter of accommodation.15–17 The present study has shown that, in the human, polygonal arrays exist as early as age 25 yr, when the human eye is capable of 7.8–12.2 diopters of accommodation.18 Interestingly, these arrays are also present at age 51–94 yr, when accommodation ranges from 2.6–0 diopters, and the distance between adjacent vertices changes little or none at all over this span of age. One speculation is that these arrays in the human may serve as an elastic inverted geodesic dome which enables the cells to change shape along with lens shape changes during accommodation. Or, if found to be associated with myosin, this actin network may exert a contractile force which moves the anterior surface of the lens into a more spherical shape during near-point accommodation. Since we have studied only the actin component of these arrays, we do not yet know if other components, for example, myosin or actin-binding proteins, are lost with age. However, the overall presence of the arrays in human lenses of advanced age argues against a positive role in the accommodative process.

In certain cultured cells, the arrays appear transitorily during respreading, preceding the formation of stress fiber bundles.1–10 It has been suggested that these polygonal networks are associated with the development of tension in a cellular sheet.1 In the intact human lens, polygonal arrays in the anterior epithelial cells do not appear to be transitory, and have been seen in all of our samples. Therefore, we conclude that the polygonal networks are a “permanent” structure in lens epithelial cells, i.e., components may turn over, but the general structure appears to remain throughout adult life, with a decrease in organization in the ninth decade.

A more plausible function, in view of the characteristics of the polygonal arrays presented here, is one of restraining the tension of the zonular pull on the epithelium during lens flattening. This tension would persist in the aging eye long after lens shape changes for near point accommodation are no longer possible in the increasingly rigid lens. Acting as a flattened Buckminster-Fuller geodesic dome, polygonal arrays may serve to stabilize the lens epithelium during zonular tension. This function of the actin network would be expected to be present in all lenses of species capable of even minimal lens shape changes.

**Key words:** human lens, epithelium, actin filaments, polygonal arrays, aging

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### References