Human lens epithelium in normal and cataractous lenses

Per P. Fagerholm and Bo T. Philipson

Epithelial cell appearance and cell size were studied in intact, unfixed, and unstained human lenses. Ten normal and 24 cryoextracted senile or presenile cataractous lenses, all with subcapsular cataract, were examined. The cell size of the transparent normal lenses showed little variation. However, in the cataractous lenses, a wide range was found in mean cell size of different lenses and variation in cell size within the individual lenses. No correlation was observed between cell size and degree of subcapsular cataract or combination of cataract type.

Key words: lens epithelium, epithelial cell size, normal human lens, subcapsular cataract, cortical cataract, nuclear cataract

The lens epithelium and its morphological changes during the development of cataract have been studied almost exclusively under experimental conditions. Most of the earlier studies of the lens epithelium in human cataracts were done with the aid of light microscopy. However, in some cases electron microscopy was used. These studies have revealed three principal changes in the epithelium: (1) signs of degeneration affecting the size and shape of cells, (2) local proliferation resulting in multilayered epithelium, and (3) proliferation along the posterior lens capsule. Ultrastructural changes include increased vacuolization, both intracellular and extracellular, and signs of degeneration.

Of the different types of human cataract, the subcapsular type has been associated with malfunction of the epithelium. Furthermore, mechanical damage of the epithelium in humans and animals can cause a progressive subcapsular cataract. In this case, the size of the epithelial defect determines the degree of subcapsular edema and cataract formation. It is most likely that a malfunctioning epithelium is the origin of the edema in senile subcapsular cataracts.

The present study aims at analyzing whether subcapsular edema is accompanied by morphological epithelial changes. The epithelial cell appearance and the cell size were studied in a large number of intact lenses with subcapsular cataracts by means of a Nikon dipping cone objective.

Materials and methods

Ten normal lenses, two from enucleated tumor-bearing eyes and eight from corneal donor eyes, were examined. All lenses were extracted atraumatically by cutting the zonulae within 12 hr after enucleation or death of the patient. The lenses were then kept in Eagle's minimal essential medium (MEM). Only lenses with no pathological opacities were studied.

Twenty-four consecutively cryoextracted senile...
or presenile cataractous lenses were classified in the slit-lamp microscope prior to operation. The presence of subcapsular cataract, cortical cataract, and nuclear cataract was graded in a manner similar to that presented by Chylack.14 All patients had a severe impairment of visual acuity, 20/200 or less in the operated eye. After cryoextraction, the lenses were immediately put into Eagle's MEM and examined within 60 min. The anterior epithelium of each lens was examined in a light microscope with a Nikon 20× dipping cone objective (Fig. 1). The lenses were unstained except in three cases; vital staining with trypan blue was tested in these lenses. Nuclei of the epithelial cells were stained in two of the cataractous lenses. The central area of the lens surface within a diameter of 7 to 8 mm was studied with reference to morphology and size of epithelial cells. The area of the cryo-probe application was recognized as a circular zone of distinct cell borders. The peripheral part of this zone showed severely damaged cells (Fig. 2). This zone was always located peripherally and was therefore excluded from this study. Photographs of the lens epithelium were taken at the anterior pole. From these photographs, the number of cells per square millimeter was determined by cell counts in areas of 0.10 to 0.15 mm². The variation in cell size was determined by a selection of 100 cells within an area of 0.2 mm². A coordinate system with 100 randomly determined coordinates was used to sample the cells. The area and the average diameter for a single cell was determined from the photographs by means of a transparent foil with circles of different diameter. The circles had the same center, and the diameters had intervals of 2.5 μm. This method was found to provide sufficient accuracy for this study. Ten of the cataractous lenses were prepared for histological examination.

**Results**

**Normal lenses.** The lens epithelium was barely visible when examined under the microscope during the first hours after enucleation of the tumor-bearing eye or after the death of the eye donor. In order to make the cell borders clearly visible, the lenses were kept at 4°C for 4 to 24 hr. The number of the cells and their shape were studied in two lenses with visible epithelium after 4, 10, and 24 hr. No significant changes were seen during this interval.

The epithelium was found to always cover the inspected anterior lens surface. The number of cells per square millimeter at the anterior pole is presented as a function of age in Fig. 3 for each of the 10 transparent lenses. The mean cell count with a 95% confidence interval x ± t0.05 for the 10 normal lenses was 3900 ± 220 cells/mm², and the corresponding mean cell area was 257 μm² (18 μm in diameter). The diameter of the epithelial cells was found to vary from 12.5 to 22.5 μm.

Two representative distributions of epithelial cell diameters are given in Fig. 4. In addition, the number of cells per square millimeter in three lenses was determined at the anterior pole and at 2 and 4 mm from the anterior pole. The results are presented in Table I.

**Cataractous lenses.** The types of cataract in the 24 lenses are given in Table II. In all lenses with posterior subcapsular cataract, the opacification covered 50% or more of the posterior subcapsular cortex. In 10 lenses, the entire cortex was opaque and vacuolized. The cortical opacities had varying distribution. The degree of nuclear turbidity and color varied in the nuclear cataracts.
Fig. 2. Lens epithelium at the site of the cryoprobe. Micrograph shows the demarcation line (dl) between frozen (f) and nonfrozen (nf) cells. (Bar = 100 μm.)

Fig. 3. Number of epithelial cells per square millimeter at the anterior pole as a function of age. o, Normal lenses; X, cataractous lenses.

The appearance of the epithelium in the cataractous lenses varied greatly. It ranged from cells having a normal appearance with barely visible cell borders to cells having cell borders that were clearly visible. The mean number of cells per unit area varied in different lenses, ranging from 550 to about 4000/mm² (Fig. 3). The corresponding variation in mean cell diameter was from 12.5 to 60 μm. The cell diameters varied greatly within the individual lenses. Three examples of the distribution of epithelial cell diameters are given in Fig. 5. The mean numbers of cells per square millimeter for different types of cataract are given in Table II. Frequently, epithelial cells were not visible in areas close to the anterior pole in lenses with dense subcapsular vacuolization (Fig. 6). In these cases, the epithelial cells could not be made visible by changing the focus or the illumination. The peripheral epithelium remained unaffected in most lenses. No correlation between cell count and the degree of subcapsular cataract or type of cataract was found. However, areas with nonvisible or absent epithelial cells were found in all 10 lenses with pronounced subcapsular edema and vacuolization. The areas with no visible epithelial cells varied from a few percent of the anterior lens surface to about 25%. Four of these lenses were prepared for histological examination. In areas of no visible cells, examination confirmed cell absence in the sections.

Discussion

In the past it had been possible to examine the lens epithelium only in intact and unfixed...
lens with the phase-contrast microscope.\textsuperscript{15} This method is very difficult to use, especially in cataractous lenses, and generally gives only a small visual field. The present method, by means of the dipping cone objective, provides a large visual field, thereby simplifying orientation as well as examination of the lens. The visibility of the cells was improved by keeping the lenses in a cold tissue culture medium. This preservation did not change the gross appearance of the cells but made the cell borders more distinct, probably because of the enlargement of the extracellular space and to the formation of extracellular vacuoles.

In normal lenses, the average number of epithelial cells per unit area at the anterior pole showed very small variations, with figures around 4000/mm\textsuperscript{2} (Fig. 3). Furthermore, no age-dependence in the cell count could be found in this material, with an age distribution from 43 to 71 years. Within the individual lens, the cell density increased toward the periphery.

In the cataractous lenses, the counts for different lenses varied greatly. A few lenses had a cell count around 4000/mm\textsuperscript{2}, i.e., of the same magnitude as the normal lenses. Most lenses had lower cell counts, with the lowest at 550 cells/mm\textsuperscript{2}. The cell size within

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1.png}
\caption{Distribution of cell diameters in the lens epithelium at the anterior pole of normal and cataractous lenses.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure2.png}
\caption{Normal lens of a 46-year-old subject.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure3.png}
\caption{Normal lens of a 69-year-old subject.}
\end{figure}
Fig. 5A. Cataract engaging the entire subcapsular cortex, and a dense, dark brown nuclear cataract from a female, age 91. Visual acuity, 20/1000.

Fig. 5B. Cataract engaging the entire subcapsular cortex, and a cortical cataract engaging all quadrants anteriorly and posteriorly from a female, age 67. Visual acuity, <20/1000.

Fig. 5C. Cataract engaging the entire subcapsular cortex, cortical cataract in all quadrants, and a light brown nuclear cataract from a female, age 66. Visual acuity, <20/1000.
Fig. 6. Light micrograph of the epithelium (e) at the anterior pole in an intact lens from a female 75 years old. The lens cortex was opaque and vacuolized. An area exhibiting an absence of epithelial cells was observed even after adjusting the focus and illumination. Subcapsular Morgagnian spheres (MS) are frequent. (Bar = 100 μm.)

Table I. Epithelial cells per square millimeter in normal lenses

<table>
<thead>
<tr>
<th>Age</th>
<th>Anterior pole (AP)</th>
<th>2 mm from AP</th>
<th>4 mm from AP</th>
</tr>
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<tbody>
<tr>
<td>46</td>
<td>3650</td>
<td>3770</td>
<td>4160</td>
</tr>
<tr>
<td>52</td>
<td>3800</td>
<td>4110</td>
<td>4350</td>
</tr>
<tr>
<td>71</td>
<td>3650</td>
<td>4180</td>
<td>4690</td>
</tr>
</tbody>
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Each cataractous lens varied extensively. The low number of epithelial cells per area could be explained by (1) inherited low number of cells per area, (2) cell degeneration and cell death, (3) reduced mitotic activity, or (4) various combinations of 1, 2, and 3.

A slight or moderate subcapsular lens edema can be explained by an insufficient epithelial transport function. The epithelial transport function can be reduced without gross morphological changes in the epithelium, as indicated by the cataractous lenses exhibiting normal cell count (Fig. 3). Areas with no visible cells were found in all lenses with substantial subcapsular edema. This could be explained either by a true absence of cells or by inability to visualize them. If the cells are absent, this would largely contribute to the observed edema in the same way as in an injured lens. It is less likely that the cells are present but not seen in these cataractous lenses, since histological examination also showed a lack of epithelial cells.

The corneal endothelium has many similarities with the lens epithelium, especially in the realm of the transport function. A low number of endothelial cells per square millimeter might be present without any detectable corneal edema. We have not yet found

Table II. Mean number of epithelial cells per square millimeter at the anterior pole in different types of cataractous lenses

<table>
<thead>
<tr>
<th>Type of cataract</th>
<th>No.</th>
<th>Mean age</th>
<th>mean cells/mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSC</td>
<td>1</td>
<td>52</td>
<td>3400</td>
</tr>
<tr>
<td>PSC + CC</td>
<td>6</td>
<td>73</td>
<td>2820</td>
</tr>
<tr>
<td>PSC + NC</td>
<td>9</td>
<td>72</td>
<td>2680</td>
</tr>
<tr>
<td>PSC + CC + NC</td>
<td>8</td>
<td>77</td>
<td>2790</td>
</tr>
</tbody>
</table>

PSC = Posterior subcapsular cataract; CC = cortical cataract; NC = nuclear cataract.
clear lenses with very large epithelial cells, but these may exist. However, it is also possible that the epithelial morphology, compared with the corneal endothelial morphology, is more correlated to function. Further studies of a large number of clear lenses are in progress.

REFERENCES