The posterior corneal surface in Fuchs' dystrophy
Scanning electron microscope study

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This study comprises 12 corneal buttons removed at the time of keratoplasty from patients with bullous keratopathy due to Fuchs' dystrophy. All except one were aphakic. Three had vitreous contact to the endothelium. One patient had several intraocular procedures. Specimens were processed for scanning electron microscopy and dried in a freeze-drying or critical-point machine. Portions of these specimens were processed for transmission electron microscopy or paraffin sectioning. Specimens can be separated in three groups based on our findings: (A) corneas with abnormal or absent endothelium; posterior surface with many fibroblast-like cells; long filaments over Descemet's membrane; and few and small warts. (B) Endothelial cells were present for the most part. Cells were of abnormal size and shape with fibroblastic cells present over or between them; no warts or filament were seen. (C) Absent or abnormal endothelial cells, with no fibroblastic cells; Descemet's layer was covered with a profuse number of warts of oval or rounded shape and of different sizes. It is believed that the fibrils seen in specimens of Group A with vitreous contact may correspond to vitreous strands. The fibroblastic cells present in many aphakic corneas could have originated by metaplasia of endothelial cells or from macrophages of uveal origin. Vitreous contact may have occurred before or after these changes in Descemet's membrane.

Key words: corneal endothelium, corneal edema, corneal dystrophy, bullous keratopathy, scanning electron microscopy, Fuchs' dystrophy, vitreous contact.

Bullous keratopathy is one of the manifestations of chronic edema and is characterized by various degrees of corneal clouding due to stromal swelling and epithelial bullae. The most common causes of bullous keratopathy requiring corneal transplantation are Fuchs' dystrophy and corneal edema following cataract surgery. This last condition may be associated with endothelial dystrophy or may be the result of operative complications or vitreous adhesion to the endothelium. It is known that excrescences or warts in Descemet's membrane (cornea guttata) precede the development of corneal edema, and bullous keratopathy frequently occurs in eyes with cornea guttata after uncomplicated intraocular surgery.
Table I. List of cases with their preoperative clinical diagnosis. Three groups (A, B, and C) are outlined for descriptive purposes (see text). The presence of warts in Descemet's membrane (guttata) as seen in cross-sections of the cornea is indicated in each case.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Clinical diagnosis</th>
<th>Age</th>
<th>Guttata</th>
</tr>
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<tbody>
<tr>
<td>A. 1.</td>
<td>Bilateral Fuchs' dystrophy, aphakic</td>
<td>70</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Bilateral bullous keratopathy, aphakic (vitreous adhesion)</td>
<td>65</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Bullous keratopathy with vitreous touch</td>
<td>70</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Bilateral bullous keratopathy, aphakic (vitreous adhesion)</td>
<td>61</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Bilateral bullous keratopathy, aphakic</td>
<td>67</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Bilateral bullous keratopathy, aphakic</td>
<td>76</td>
<td>+</td>
</tr>
<tr>
<td>B. 7.</td>
<td>Monocular aphakic bullous keratopathy</td>
<td>64</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Monocular bullous keratopathy, aphakic</td>
<td>70</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>Bilateral bullous keratopathy, aphakic</td>
<td>67</td>
<td>-</td>
</tr>
<tr>
<td>C. 10.</td>
<td>Bullous keratopathy, aphakic</td>
<td>74</td>
<td>+++</td>
</tr>
<tr>
<td>11.</td>
<td>Bilateral bullous keratopathy, aphakic</td>
<td>62</td>
<td>+++</td>
</tr>
<tr>
<td>12.</td>
<td>Bilateral bullous keratopathy, phakic</td>
<td>61</td>
<td>+++</td>
</tr>
</tbody>
</table>

Since the original ultramicroscopic study of “cornea guttata” by Feeney and Garron in 1961 and by Jakus in 1962, this entity has been the subject of several reports here and abroad. Except for two publications, the surface morphology of cornea guttata in Fuchs’ dystrophy as seen with the scanning electron microscope (SEM) has not been well studied.

Cornea guttata and Fuchs’ dystrophy are terms interchangeably used by clinicians when corneal edema develops. Warts in Descemet’s layer are one of the manifestations of the dystrophic endothelium which may go on for years before it fails to maintain the cornea deturgesced, or before the dystrophy decompensates. Endothelial dystrophy with abnormal Descemet’s layer without warts is another manifestation of Fuchs’ dystrophy not usually recognized. The presence of isolated warts in this manifestation of Fuchs’ dystrophy, or warts buried in fibrillar basement membrane-like material indicates that they are different manifestations of the same disease. Vitreous adhesions to the dystrophic endothelium are likely to occur in these cases; however, similar histologic alterations are found in the endothelium after chronic vitreous adhesion. The sequence of events must then be evaluated together with the clinical history and examination of the other eye.

The purpose of this study is to demonstrate the alterations in the posterior corneal surface of corneas with bullous keratopathy of varying degrees due to Fuchs’ dystrophy, some of them with vitreous adhesion, as seen with the (SEM).

Materials and methods

This study was done on twelve corneal buttons (7 to 7.5 mm.) removed at the time of keratoplasty by myself or Dr. H. E. Kaufman. Eleven patients had various degrees of bilateral aphakic bullous keratopathy, except for two patients who had a normal contralateral cornea to slit lamp examination. One patient had cornea guttata with moderate edema and was phakic in both eyes (Table I).

As soon as the corneal discs were removed from the patient they were immediately placed in cold 4 per cent glutaraldehyde where they remained for a period of time from eight to 72 hours. A small segment of the corneal disc was removed for light or transmission electron microscopy and the remaining tissue was processed for SEM. This tissue was rinsed in distilled water, postfixed in 1 per cent osmium tetroxide for 30 minutes, and again rinsed in distilled water and run through a graded series of ethyl alcohols. Once in 100 per cent alcohol it was dehydrated in freeze-drying or in a critical-point machine. Thereafter, the specimen was mounted on an aluminum stub and coated with gold palladium. Specimens were examined in a scanning electron microscope (Stereoscan, Cambridge, England) at 10 or 20 KV. Pictures were recorded on Polaroid P/N film.

Paraffin specimens were processed in a standard way and stained with hematoxylin and eosin and periodic acid-Schiff (PAS). Transmission electron microphotographs were taken with a Zeiss 9-S2.
Fig. 1. SEM of the endothelial surface of a cornea with bullous keratopathy showing an abnormal cell surface and multiple fibroblast-like cells randomly oriented. (SEM, x200.)

Fig. 2. Microphotograph of an area of the previous picture at higher magnification. Abnormal endothelial cells (E) are seen together with fibroblastic cells. The endothelial cells are broad and elongated with serrated borders and filamentous prolongations over Descemet's membrane (D). This layer is covered by fibrinous material. At times these endothelial cells seem to take the shape of fibroblastic cells (arrow). (SEM, x1,000.)

Fig. 3. Another area showing what appears to be transitional figures of flat endothelial cells to fibroblastic elements. Debris in the form of amorphous clumps are seen between cells of Descemet's (D) membrane. (SEM, x1,000.)

Fig. 4. High-power microphotograph of an endothelial cell (E) with microvilli and cytoplasmic filaments which make contact with fibroblastic cells. Descemet's membrane (D) is covered by fine filamentous material. (SEM, x5,000.)

Fig. 5. Microphotograph shows the surface of Descemet's membrane (D) with nodular excrescences (arrow) and absence of endothelial cells. Filamentous deposits are seen spreading over the surface, at times forming clumps. (SEM, x1,000.)

Fig. 6. When these long filaments are examined at higher magnification, they appear like a fine brush laid over the rough-surfaced Descemet's membrane. (SEM, x2,000.)
electron microscope after the specimen had been cut into small pieces and postfixed in 1 per cent osmium tetroxide with phosphate buffer for 90 minutes. After dehydration through a graded series of alcohols, specimens were finally embedded in epon.

Results

Based on our histologic findings, specimens can be divided into three main groups (Table I):

A. Corneas with abnormal or absent endothelium. Fibroblast-like cells present in small or profuse number over abnormal Descemet's membrane. Occasional small warts. Thick fibril deposition over Descemet's membrane and warts. Thick or laminated Descemet's membrane with warts in cross-sections. Thickness, 12 to 16 microns.

B. Endothelial cells present for the most part. Cells of abnormal size and shape. Fibroblast-like cells present over or between endothelial cells. No warts or long filaments. In cross-sections, Descemet's membrane appears thick with overlying fibrous membrane. Wavy or laminated at times. Thickness, 18 to 28 microns.

C. Endothelium absent in many areas. Multiple warts of round or oval shape and of different sizes. Descemet's membrane of irregular thickness with numerous warts. Thickness varied from 12 to 18 microns.

Group A. Comprises six cases of advanced bullous keratopathy, three of them with vitreous contact to the endothelium. Case 1 showed a profuse number of fibroblast-like cells scattered and randomly oriented over the endothelial surface. Normal endothelial cells, however, could not be identified. Survey of the graft posterior surface showed that the cellular elements present were flat and elongated with serrated borders or long lateral prolongations which interconnected with each other (Fig. 2). They formed monolayers, although occasionally more than one layer of cells were seen, some of them apparently transforming to spindle-like cells (Fig. 2, arrow). Large areas were devoid of cells as shown in Fig. 3 (D); but the surface of Descemet's membrane was covered by a layer of fibrin-like fibrillar material. Fig. 4 shows, at higher magnification, the surface of a fibroblast-like cell next to a flattened cell to which it is in contact by means of cytoplasmic processes. Fig. 5 and 6 show the corneal endothelial surface of an eye with severe postcataract bullous keratopathy of long standing (Case 2). Cross-sections showed a Descemet's layer of 15 to 22 microns in thickness with occasional small guttata and absence of endothelium. In SEM specimens, endothelial cells could not be identified; Descemet's membrane was covered by amorphous material of rough texture as in the previous case and there were a few small warts. Long filaments were deposited on its surface and over the warts (Fig. 5). In some areas they were clumped together, whereas in other areas the filaments spread out like brushes over large areas (Fig. 6). Similar findings were observed in three other cases. The one illustrated in Figs. 7 through 11 showed occasional abnormal cells over Descemet's membrane which had few warts of small size. Fibrin-like deposits were not observed but there were large numbers of filaments randomly distributed over the corneal surface (Figs. 7 and 8). Vitreous adhesion to the posterior cornea was present at the time of surgery and vitreous fibers were found in some areas of PAS-stained cross-sections of the cornea (Fig. 9). It is possible, therefore, that these filaments and those seen in other cases may correspond to vitreous fibers or strands.

Group B. Figs. 12 and 13 correspond to an eye with bullous keratopathy following surgery for congenital cataracts (Case 7) 20 years previously and multiple intraocular procedures. Cross-sections examined with the transmission electron microscope showed a thick (24 to 28 micron) Descemet's layer in which Descemet's membrane material alternated with fibrillar and fibrous tissue. Fibroblast-like cells were present in the membrane and over and between endothelial cells (Fig. 12). SEM microphotographs showed areas of absent or abnormal endothelium. Endothelial cells
of irregular shape intertwined with fibroblast-like cells. At times these were partially visible under the endothelial layer (Fig. 13). Similar findings were observed in another case with bilateral corneal disease.

Case 9 differed somewhat from the previous case in which the corneal edema had appeared only two years after uncomplicated cataract extraction and it was of moderate degree. Low-power microphotographs showed a complete endothelial mosaic (Fig. 14) with variations in size and shape frequently found in this age group. Guttata excrescences were not seen but there were rows of fibroblast-like cells on their surface. These cells were similar to those previously described, except that these had a cellophane-like expansion on one or both ends to which other cells attached. In contrast to previous specimens, groups of these cells seemed to be oriented in the same direction. Endo-
Fig. 12. Microphotograph showing a fibroblastic cell (Fib.) over Descemet's membrane. This cell is partially covered by endothelial cells (En) (arrow). (SEM, ×5,000.)

Fig. 13. Transmission electron microphotograph of a section of the same cornea. It shows a thick Descemet's layer (Des.) and a cross-section of a fibroblastic cell which has characteristics of a macrophage. Arrow shows rest of endothelial cell (En). (×12,000.)
Fig. 14. Specimen obtained at the time of keratoplasty shows an intact endothelial layer covered by multiple fibroblastic cells, most of them oriented in one direction and arranged in chains. Other areas did not show such cells. (SEM, ×500.)

The endothelial cell size varied between 10 and 30 microns. Nuclei were of similar size and cell borders had tight junctions. Most of the cells had microvilli and one or two long villous filaments (Fig. 15). Frequently, these were located over the nuclei and showed a cytoplasmic thickening at its base where the filament also became thicker. This structure was often in contact with or partially wrapped around the fibroblast-like cell. PAS-stained sections showed a thickened Descemet's membrane with lamination as well as flat endothelial cells. Cross-sections of fibroblastic cells were found frequently. Transmission electron microscopy also showed a thick laminated Descemet's layer with thin endothelial cells over fibrillary material, but no fibroblastic cells could be found in these specimens.

Group C. Figs. 16 through 18 correspond to Case 10 and demonstrate the appearance of a cornea with numerous guttate formations which can be easily visualized in some areas due to the absence of endothelial cells. Absence of endothelial cells is, in part, artificial as determined by the presence of cell residues; in other areas it seems to be due to actual cell degeneration. The artifact shown in Fig. 17 facilitates the observation of a wart in Descemet's membrane under the ruptured cytoplasm of an endothelial cell. In many areas these cells are totally absent revealing the surface structure of Descemet's membrane warts (Fig. 18). These warts vary in size from 5 to 25 microns or more. Their shapes vary from round to oval, often two warts are joined by a solid bridge. The top was usually flat but some warts had a slightly dimpled or umbilicated surface. Most of the times they had steep borders, occasionally they showed sloping sides, but warts with undermined sides, as would be expected in collar-button warts, could not be found. Descemet's membrane surface had a fibrillar surface which, in a whorl-like fashion, circled the warts. They probably corresponded to fine collagen fibrils (long spacing) found around the warts in transmission electron microscopy specimens. Fig. 19 corresponds to Case 12 which was phakic. It shows several warts with a flatter surface surrounded by fibrillar material.

Comment

Fuchs' corneal dystrophy is characterized by primary changes in the corneal endothelium and in Descemet's membrane. It has been shown that endothelial cells acquire the morphology and the function of fibroblasts while they produce basement membrane-like material characteristic of...
endothelial cells and collagen fibrils typically produced by fibroblasts. The distribution of this newly produced material over the original Descemet's layer will originate the typical warts, the thickened Descemet's membrane, or the intermediate forms we have discussed here. Endothelial cells eventually degenerate and leave a bare fibrillar surface, or a more compact Descemet's wart.

Absence of endothelial cells is a frequent finding in this condition. The large areas without endothelium seen in our first cases could have been due to artifacts, however, care was taken not to disrupt vitreous adherent to the endothelial surface. The long fibrils present in several cases of aphakic bullous keratopathy suggest that they correspond to vitreous fibers or strands. They could not be demonstrated in transmission electron microphotographs nor have they been described before, but they have been seen frequently in PAS-stained sections.

Our SEM microphotographs support the view that endothelial cells may transform into fibroblasts because, in addition to the deposition of collagen material, they
have cytoplasmic fibrils, coated vesicles, prominent cytoplasmic processes, increased RER, and desmosomes.

As mentioned before, transmission electron microscopic studies of endothelial cells in Fuchs' dystrophy suggest that these cells transform into fibroblasts. Our SEM studies support this view on a morphologic basis as seen in Figs. 1 through 4, corresponding to Case 1. We have seen, in others cases of Fuchs' dystrophy, what appear to be transitional figures from flat endothelial cells with prominent cytoplasmic processes to spindle-shaped processes with one or both ends tapered. Since these cells have been found only in aphakic eyes (only one phakic eye case was studied) it is possible that some fibroblastic cells are actually fibroblasts or macrophages of uveal origin, and that the vitreous plays a major role in their access to the cornea and in their proliferation or transformation from endothelial cells to fibroblasts. Fibroblastic cells have been seen in regenerating endothelial injuries, in retrograft membranes, and after chemical injuries. Once the stimulus for fibroblastic cell activity or transformation disappears, these cells may revert to their original endothelial aspect. We have seen such fibroblastic cells in SEM specimens of retrocorneal membrane formation and graft failures and in clinical specimens of rejected grafts.

Cross-sections of specimens described in Group B show the structure of those cells which appear to be the fibroblasts seen in SEM pictures. Their internal structure seems to correspond to macrophages in two cases.

We found great variations in the thickness of Descemet's membrane between cases (16 to 28 microns) and in the same specimen. The thicker membranes did not show guttate formations in SEM specimens and in transmission electron microscopy sections they were small and buried in fibrous or basement membrane-like material. The severe degree of pathology seen in cross-sections did not correspond to the almost normal appearance of the endothelial surface which showed abnormal cells and fibroblasts on their surface. These are the corneas which may appear normal by slit lamp examination before they decompensate following cataract surgery and possibly be used as normal donor material. Stocker has called attention to this little recognized aspect of Fuchs' dystrophy which has important clinical significance. Guttate formations, on the other hand, are early and easily recognized because they produce a typical morphologic change in the posterior corneal surface. Probably, this is a more severe type of disease, but not all patients with cornea guttata develop edema. Even though the morphology of Descemet's membrane with multiple warts does not add new information to the pathogenesis of this disease, it helps to understand the magnitude of the disease, particularly when the warts can be seen without their endothelial cover.

H. E. Kaufman, M.D., J. Chandler, M.D., P. Binder, M.D., and R. Abel, M.D., contributed with specimens. Kenneth Faust, M.D., referred two cases included in this study. The collaboration of T. Sakimoto, M.D., is acknowledged as well as the technical assistance of W. M. Chisholm, J. Valenti, and E. J. Jenkins. Comments to a portion of this study were made by Professor G. K. Smelser. Dr. E. Balazs gave comments about some photographs.

REFERENCES


