Scanning electron microscopic studies on the development of the iridocorneal angle in human eyes

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The structural changes occurring in the iridocorneal angle of human eyes from fetuses, premature and mature infants, and adults were studied with light and electron microscopy. The scanning electron microscopic technique with the use of freeze-dried tissue pieces provided much new information. The iridocorneal angle is covered by a continuous monolayer of polyhedronal endothelial cells up to about eight months gestation age. The uveal meshwork consists of two parts, each of which has its own characteristic structure. The endothelial cells in the iridocorneal angle change their shape and become separated by holes of varying sizes. They are observed to be arranged as sheaths in several layers. The trabeculae in the adult eye are covered with multipolar endothelial cells arranged in several layers. The lamellar structure of the corneoscleral trabeculae is observed readily on scanning electron micrographs. The results obtained are discussed in relation to previous studies on the iridocorneal angle with light and transmission electron microscopy. The possible role of a persisting continuous endothelial membrane in the iridocorneal angle for the pathogenesis of congenital glaucoma is stressed.

Key words: human eye, iridocorneal angle, iris, cornea, endothelial membrane, endothelial cells, trabeculae, development, scanning electron microscopy, transmission electron microscopy, congenital glaucoma.

Different opinions exist about the structure and structural changes occurring in the iridocorneal angle of human beings during different stages of development. A question which has caused much discussion is whether there exists any continuous lining of endothelial cells, arranged as a membrane, in the iridocorneal angle in fetuses and, in such a case, whether this thin monolayer is of importance for intraocular pressure during postnatal life. Clinical evidence speaks in favor of the opinion that there exists a continuous endothelial monolayer covering the iridocorneal angle and that it may persist as a developmental anomaly causing congenital glaucoma.

The aim of the present study is to examine whether any continuous lining of the primitive anterior chamber by endothelial cells does exist in human fetuses and to study the developmental changes of the iridocorneal angle. The combined use of
phase interference microscopy according to Nomarski, transmission, and, especially, scanning electron microscopy affords possibilities to observe details in the structure of the iridocorneal angle and to evaluate the results obtained with special regard to the artifacts induced by the preparative techniques in the delicate fragile structures of this region.

Material and methods

Preparation of material. Human fetuses of varying ages and with known crown-rump length were obtained within a few minutes after the completion of legal abortions. Eyes, enucleated due to orbital or intraocular tumors, from adult human patients, were obtained from the Department of Ophthalmology, University of Goteborg. Eye bulbs were also obtained at autopsies of human beings of both sexes. The age of the patients varied from premature infants to 63 years old. The time elapsing between the death and the preparation of specimens varied from a few hours up to two days, although great care was taken to reduce this time period as much as possible.

The desired areas from the enucleated eyes were dissected and frozen quickly in isopentan-propane chilled with liquid nitrogen, either unfixed or after fixation in a cacodylate-buffered solution containing glutaraldehyde, formaldehyde, and calcium. Other specimens were prepared for light microscopy (stereo microscopy and phase interference microscopy according to Nomarski) and transmission electron microscopy. Tissue pieces were also examined in the scanning electron microscope after air-drying or after dehydration in acetone or ethanol.

Results

General considerations. The scanning electron microscopic studies on freeze-dried tissue pieces provide most of the new information and will receive, therefore, the most attention. Specimens, prepared from the same eye but in different ways, proved that the most reproducible results were obtained from unfixed or fixed freeze-dried samples. Air-drying or dehydration with acetone or ethanol followed by air-drying always resulted in uneven shrinkage, distortion, and other artifacts to an extent never observed in properly treated, freeze-dried specimens. The fixation technique used seemed to induce slight changes in the dimensions of the endothelial cells and fibers as compared to the results obtained from unfixed, rapidly frozen specimens and from those specimens observed with the aid of the Nomarski light microscope. Consequently, the results described here are based upon scanning electron microscopic studies of freeze-dried tissue pieces.

Iridocorneal angle in eyes of fetuses, premature infants, and children. The primordium of the anterior chamber was closed off from the posterior parts of the eye bulb by the iridopupillary lamina up to 6 fetal months. The lumen was small and lined by endothelial cells.

The iridocorneal angle was covered by a continuous thin monolayer of endothelial cells in premature infants up to about eight months of age (Figs. 1 to 6). The endothelial cells at the periphery of the cornea were polyhedronal (often hexagonal) and of fairly uniform size and shape. The endothelium covering the developing corneoscleral trabecula differed from that lining the cornea. The former cells were larger and flatter. The border between adjacent cells looked more irregular and less distinct than the one between the endothelial cells on the cornea (Figs. 1 to 4). All of the endothelial cells in the iridocorneal angle showed short processes along their border, interdigitated with those of neighboring cells (Fig. 5). A small number of microvilli were observed on most of the endothelial cells. These delicate structures became detached easily during the preparation of the specimens or were masked by amorphous precipitates.

The complicated structure of the meshwork in the iridocorneal angle at this stage of development, i.e., up to about eight fetal months of age, was easy to observe with
scanning electron microscopy of freeze-dried specimens, as well as with the Nomarski light microscope. The meshwork beneath the monolayer of endothelial cells appeared denser and more regular in its organization than the deeper lying uveal meshwork (Fig. 6). Characteristic features of the former were the sheaths of thick fibers running parallel to the iridocorneal angle, recognizable in specimens from 16-weeks-old fetuses.

The appearance of the iridocorneal angle became thoroughly changed during the last weeks before and the time following birth. The continuous covering of endothelial cells was split along the border between neighboring cells (Figs. 7, 12). There was no significant increase in the number of impressions in the surface of the endothelial cells. The size and the number of the intercellular slits increased rapidly. These developmental changes were not uniform in the iridocorneal angle of eyes of the same age, not even in the same eye. Figs. 7 and 8 are both from the same eye, but different sectors, and support the commonly made observation in the scanning electron micrographs. Concomitantly, there was a marked increase in the number of fibrils and their size. This meshwork was covered by endothelial cells and processes with the result that the endothelial cells became separated from each other. Shortly thereafter, single endothelial cells were observed in several different planes in the iridocorneal angle following the course of
Figs. 2 and 3. (Fig. 2) The endothelial cells on the cornea (CE) are seen as polyhedronal cells in the upper part of the figure and those on the corneoscleral trabecula (TE) below. The border between these two different cell types is marked by arrows. Human premature infant with a crown-rump length of 26 cm. and an estimated age of 30 weeks. (Original magnification x1,160.) (Fig. 3) Higher magnification of the central area in Fig. 2 showing the fairly distinct border (arrows) between the corneal (CE) and the trabecular (TE) endothelial cells. Note the impressions in the latter cells which are usually larger and flatter than the former. (Original magnification x2,350.)
the fibers in the iridocorneal angle. It was often difficult to differentiate cell processes from fibers on the scanning electron micrographs (Figs. 7 to 12). No definite signs of degeneration could be observed in the endothelial cells in the iridocorneal angle on the transmission electron micrographs. The endothelial cells in the different layers...
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Fig. 6. The uveal meshwork in the picture consists of an anterior, denser part with threads (arrow) arranged parallel to the anterior chamber trabeculum iridale (Ti) and a posterior, looser ciliary portion (Tc). The corneal endothelial (CE) cells may be observed in the upper left corner. Specimen as in Fig. 2. (×3,300.)

were of identical or similar appearance when examined in thin sections.

The iridocorneal angle was covered at birth by endothelial cells forming a discontinuous layer of cells at different depths. Most of the fibers were covered by cells and cell processes.

The iridocorneal angle in eyes of adults. The corneoscleral trabecula was covered by a discontinuous layer of endothelial cells. They were star-shaped and had many long processes. Many of the trabeculae close to the anterior chamber were ensheathed, thereby, to a large extent by the endothelial cells. Slits and holes of varying sizes and shapes were formed between the meshwork of endothelial cells, their processes, and the fibers in the iridocorneal angle (Figs. 13 to 15). It was often possible to observe a complicated pattern of holes in the depth of large cryptlike structures in the surface layer (Figs. 13 to 15).

This was due to the fact that the corneoscleral trabecula was formed by layers of fenestrated lamellae. This architecture is evident in Fig. 16, showing the iridocorneal angle in an adult human eye in cross section.

The border between the angle structures and the iris root was not distinct. The anterior base of the iris was covered by endothelial cells with intercellular slits of varying sizes and shapes. Crypts, extending deep into the iris stroma, were often noticed close to the angle (Figs. 13 to 15).

Discussion

The primitive anterior chamber of the eye in human fetuses is lined during the early part of development by a continuous monolayer of endothelial cells. These cells become flattened and more irregular in the iridocorneal angle at the age of about seven or eight months in utero, thereby...
Fig. 7. The endothelial cells covering the iridocorneal angle are separated from each other during the last weeks before birth. Note that the endothelial cells (E) are growing in several layers, partly on the surface of each other. Some of the impressions (arrows) were increased in size perhaps during the preparation of the specimen. The arrowhead points on a break induced by the electron beam. (I) Iris; (C) cornea. Premature infant. (x500.)

marking the anterior border of the corneo-scleral trabeculae against the cornea. Thereafter, slits and holes appear in increasing number between adjacent endothelial cells, concomitant with a marked increase in size and number of the fibers in the iridocorneal angle. Eventually, the trabeculae and the fibers form a complicated meshwork, which is covered against the anterior chamber by multipolar endothelial cells arranged partly in several layers. This is the appearance of the iridocorneal angle at birth and the time thereafter in the normal human eye. It seems likely that the main reason for the splitting of the initially continuous monolayer of endothelial cells may be that the anterior parts of the eye increase very much in size during this time period as compared to the other parts of the eye bulb. This conclusion is supported further by the observations that the endothelial cells do not show any significant signs of degeneration when examined in the transmission electron microscope. There is no significant evidence, as far as judged from the present study, supporting the hypothesis that the discontinuity of the iridocorneal angle is due to degeneration and not redistribution of the endothelial cells.

The endothelial cells in the primitive
anterior chamber are all polyhedronal initially (chiefly hexagonal) and bulge into the chamber with their perinuclear cytoplasm. The cells in the iridocorneal angle change their size and shape at an early stage. This means that there is a distinct border between the corneal endothelial cells on one hand and those in the angular region on the other is formed. It is reasonable to suppose that this border represents a primordium of the line of Schwalbe. There is no such sharp transition in the morphology of the endothelial cell in the iridocorneal angle against the iris.

The endothelial cells in the iridocorneal angle become enlarged and more irregular in their shape with time. Processes, initially lacking completely, are seen arising from the cell body in increasing number. The initially polygonal cell thus acquires a multipolar or star-shaped cell body with no structural resemblance to the other endothelial cells lining the corneal angle. A similar drastic change in the morphology of endothelial cells has been described previously by Vrabec.11

The endothelial cells in the fetal iridocorneal angle show short interdigitating processes and folds. They are of the same appearance as those on the inner corneal...
Figs. 9 and 10. The iridocorneal endothelial membrane (E) is rebuilt during the development in such a way that it is divided into processes separated by large open impressions (arrow) reaching the line of Schwalbe (S). The bottom of the latter looks like an open meshwork partly covered by endothelial cells (arrowhead). The trabeculae (t) consist of fibers surrounded by endothelial cell processes. Premature infant as in Fig. 8, but opposite eye. (×350 and ×860, respectively.)
Figs. 11 and 12. (Fig. 11) The impressions or pores (arrow) in the corneoscleral trabecular meshwork reach the line of Schwalbe (S) between the persisting remnants of the endothelial membrane, which develop later into the iridal processes. Note the trabecula (t). The impression in the lower right corner is partly covered by an endothelial cell (E) with an erythrocyte on its surface. Same specimen as in Fig. 8. (x860.) (Fig. 12) The future iridal processes (Ip), which insert into the line of Schwalbe (S), are formed by fibers and cells coming from different levels forming a complex pattern of interwoven fibers and processes (center). The corneoscleral trabecular meshwork may be seen in the bottom of the impressions (arrow). (C) Cornea. Premature infant. x860.
Figs. 13 and 14. The complex structure formed by endothelial cells (E) and fibers arranged as trabeculae (t) is seen in this picture of the iridocorneal angle of an adult man. The line of Schwalbe is in the lower left corner of Fig. 16. The iridal processes (arrow) traverse the angle far above the trabeculae and endothelial cells. Note that the endothelial cells are multipolar and have several dividing processes of varying sizes, shapes, and lengths. The light roughly spherical particles on the surface of the angle structures are blood cells which are observed in an increased number in cadaver specimens. (×350 and ×860.)
Figs. 15 and 16. (Fig. 15) There is a considerable variation in the structure of the iridocorneal angle, not only between different human beings, but also with age. Note the fairly broad clumsy processes in the angle. The endothelial covering is seen to extend in the depth of the impressions. The cornea (C) is to the left and the iris (I) is in the upper left corner. One-year-old infant. (X 1,750.) (Fig. 16) This picture shows the anterior part of a cross-sectioned iridocorneal angle. The cornea with its lamellae of fibers and cells is seen in the lower right and the spongy corneoscleral trabecular lamella (T) in the upper left. The arrow points to a superficial trabecula. (S) Schwalbe's line; (D) Descemet's membrane. (X 910.)
surface, both in human fetuses, as shown in this study, and in rabbits, as shown previously. The subsequent loss of these characteristic cell border structures of the endothelial cells in the iridocorneal angle and on the anterior surface of the iris (Hansson, unpublished observations) reflects the morphologic adaptive changes occurring during rapid and strong development of the anterior part of the eye starting during the last fetal weeks. It is likely that these structural changes reflect the newly established demands on the endothelial cells in the iridocorneal angle and on the iris. The endothelial cells on the cornea have, as one of their functions, to take part in the regulation of the transport of fluid and salts to or from the anterior chamber, while a corresponding function for the endothelial cells in the iridocorneal angle and on the iris is to allow a relatively more unrestricted passage of fluid and salts. The structural alterations of the endothelial cells during the late fetal development form a probable adaptation to the existing functional demands in such a case.

Spencer, Alvarado, and Hayes published scanning electron microscopic pictures of the angular region of fixed human eyes recently. They described details of the iridocorneal angle with special regard to the endothelium and the trabecula. Their results agree with those obtained in the present study, as well as with other methods; however, there are several important differences. The preparative procedure used herein, i.e., freeze-drying of either fixed or unfixed tissue pieces, caused only slight if any changes in the dimensions of critical structures such as the endothelial cells, trabeculae, and iris processes when comparing the light micrographs with the use of Nomarski optics before and after fixation, freezing, and drying. This contrasts to the drastic uneven changes induced by acetone or ethanol dehydration or air-drying during the preparation of pieces of brain tissue. It ought to be mentioned, when discussing possible artifacts induced during the treatment of the specimens, that the endothelial angular membrane almost always becomes disrupted. Thus, the best method seems to be rapid freezing followed by freeze drying.

Several fibers in the iridal trabeculae are parallel and follow the contour of the iridocorneal angle. Short thin fibrils are arranged as a meshwork between the main fibers. This architecture contrasts to that of the ciliary trabeculae lying immediately behind. The latter seems to consist of a fairly irregular loose meshwork of quite coarse and short fibers. The differences in structure between the iridal and the ciliary trabeculae, observed in the scanning electron micrographs, correspond to those described previously in light and electron microscopic studies. Thus, there is good correlation between data obtained with different methods.

It has been proposed that a persisting continuous endothelial membrane in the iridocorneal angle may obstruct the aqueous outflow, thereby causing glaucoma. Gonioscopic studies support the opinion that persisting fetal tissue may be represented by a transparent membrane in the angle of the anterior changes. However, the existence of such a structure has been denied as discussed recently by Manschot. The present study has demonstrated that the fetal iridocorneal angle is covered by a continuous endothelial membrane and that this membrane is split during the last weeks of the fetal period. It seems reasonable to suppose that the splitting and rebuilding of this membrane may be arrested and thereby cause a block which prevents the normal outflow of aqueous humor through the iridocorneal angle more or less completely.

REFERENCES