Scanning electron microscopy of the trabecular meshwork in normal and glaucomatous eyes

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The ultrastructure of trabeculectomy specimens obtained from enucleated eyes was compared to that of 23 similar specimens extracted from glaucomatous eyes. It was thus possible to study the trabecular meshwork from Schwalbe’s line to the iris root, the corneal endothelium, the canal of Schlemm, its inner and outer walls, and the collectors. In the glaucomatous specimens it was possible to verify, for the first time, two aspects which show clinical correlation. The first aspect is pseudoexfoliation: we find in the trabecular meshwork two types of deposits in great quantity. One is formed by a dense irregular fibrillar net, with a diameter oscillating between 600 and 830 Å, surrounding the trabeculae; the others are pigment granules. Both fill the intertrabecular spaces. The first type is also observed on the corneal endothelium lining Schwalbe’s ring, stressing the visibility of the intercellular spaces. The second aspect is the pectinate ligament (mesodermal remnant) in late-onset congenital glaucoma. Its pattern is characteristically different from that of the corneoscleral meshwork.

Key words: trabecular meshwork, scanning electron microscopy, uveal meshwork, corneoscleral meshwork, canal of Schlemm, external collector of the canal of Schlemm, pseudoexfoliation, late congenital glaucoma, pectinate ligament (mesodermal tissue).

In previous papers we presented the light microscopic study of trabecular specimens obtained from glaucomatous eyes by trabeculectomy. We continued this research, studying the meshwork with scanning electron microscopy. At present, each specimen of trabecular meshwork is divided into three equal parts: one for light microscopy, a second for scanning electron microscopy, and a third for transmission electron microscopy.

Material and methods

Twenty-three trabecular meshwork specimens obtained by trabeculectomy from glaucomatous eyes were examined. We consider that meticulous surgical technique and careful manipulation of the piece of trabecular meshwork during its excision are very important. Transillumination, as described by Minsky, is essential to place the incision at the level of the corneal endothelium immediately beyond Schwalbe’s ring. This technique allows us to section the cornea in the area of light, at 1 mm. from the limit of light and darkness. After dissection of a limbus-based scleral flap, Minsky’s maneuver allows us to place the first incision parallel to the limbus. We section only half the thickness of the cornea.

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Fig. 1. Fibers of the trabecular meshwork. A, a, Scleral septum; b, scleral spur; c, Schlemm's canal; 1, scleral trabecular meshwork; 2, scleral trabecular meshwork coming from Brücke's muscle; 3, uveal meshwork. B, A panoramic view of the chamber angle, Schlemm's canal, and trabecular meshwork taken from an eye which had been enucleated because of a malignant melanoma. C, Schematic drawing of Fig. 1, B. a, Scleral septum; b, scleral spur; c, Schlemm's canal; d, lamina iridociliaris; e, circular circular muscle (Müller); f, major arterial circle of the iris; 1, scleral trabecular meshwork; 2, fibers of the longitudinal muscle (Brücke); 3, uveal meshwork.

Before opening the anterior chamber we mark the lateral incisions, also sectioning half the thickness of the scleral wall. After opening the anterior chamber we complete the first incision with an angled Vannas' scissors (Storz). With the same instrument we complete the lateral incisions perpendicular to the limbus. The trabeculectomy piece is gently grasped with fine-toothed microsurgical forceps (Grieshaber) by the external half of its thickness in order to avoid damaging the delicate structure of the meshwork. In this moment, the chamber angle is exposed to the surgeon's eye, and the final incision is performed with Vannas' scissors between the scleral spur and the ciliary band.

The trabeculectomy specimen is immediately fixed by immersion in 2.5 percent glutaraldehyde in 0.1M Millonig's phosphate buffer with glucose, pH 7.4, for about 24 hours. After being rinsed in isotonic buffer for 15 minutes, the specimen is postfixed in 1 percent osmium tetroxide in Millonig's phosphate buffer with glucose, pH 7.4, for 2 hours. Thereafter the sample is dehydrated in graded acetone solutions and dried by the critical-point method in fluid CO2 with a Sorvall Critical-Point Drying System. This method allows the substitution of acetone by carbon dioxide in a high-pressure chamber.

Once dried, the trabeculectomy piece is mounted on a specimen holder, with the trabecular mesh-
work facing upward, and coated with a thin layer of carbon and gold palladium in a Jeol vacuum-coating unit. The piece is examined in a Jeol JSM-U3 scanning electron microscope.

Results

Fibers of the trabecular meshwork. Fig. 1, A, shows the three different parts of the trabecular meshwork. Arrow 1 (scleral trabeculae) represents the fibers which go from sclera to sclera, from the scleral spur to the scleral septum near its end in Schwalbe's line. They form an arched bundle enclosing the canal of Schlemm and they are separated from the canal by the porous tissue.

Arrow 2 represents the fibers which form the insertion tendon of the ciliary muscle passing over the scleral spur as a bridge
Fig. 3. Outer (A and B) and inner (C and D) walls of Schlemm's canal. A, Opened external collector, diameter 50 \( \mu \) \( \times \) 1,000; B, two endothelial cells with nuclei corresponding to the inset of Fig. 6, A (\( \times \) 3,000); C, long endothelial cells, length 90 \( \mu \), width 7 \( \mu \) (\( \times \) 1,000); D, nuclei of the endothelial cells of Fig. 6, A (\( \times \) 3,000).

to insert in Schwalbe's line. They constitute the tendinous continuation of the muscular fibers of the ciliary muscle.

Arrow 3 (uveal trabeculae) represents the fibers which originate at different levels between Schwalbe's line and the scleral spur and end at the iris at different levels from the insertion of the iris root up to the iris crests. They form the uveal trabecular meshwork and are, in fact, remainders of the pectinate ligament (also known as mesoderm remnants or iris processes).

Fig. 1, B, is a scanning electron micrograph of a trabeculectomy specimen excised from an eye before enucleation necessitated by a malignant melanoma. In this case, unlike the usual trabeculectomies, the posterior section passes through the iris root.

Fig. 1, C, is a schematic representation of Fig. 1, B. In each of these figures, the left-hand third shows a section of the chamber angle and the right-hand two thirds show the chamber angle as it is evidenced by gonioscopy. The sclera emits two prongs:
Fig. 4. A to C, Opened Schlemm's canal after trabeculotomy: 1, outer wall of the canal of Schlemm exposed by trabeculotomy; 2, prominent scleral spur; 3, internal wall of the canal of Schlemm and trabecular meshwork rolled over itself; 4, anterior surface of the iris; 5, ciliary processes. Inset: two external collectors subdivided by septa.

the scleral septum (a), of which the apex corresponds to Schwalbe's ring, and the scleral spur (b). These two prongs form a furrow in which Schlemm's canal is lodged; d marks the lamina iridociliaris of Rohen (the ciliary band of the gonioscopic image); f is the major arterial circle of the iris and (e) the internal surface of the ciliary muscle (circular or Müller portion of the ciliary muscle); 1 marks the scleral trabecular meshwork, 2 the insertion tendon of the ciliary muscle, and 3 the uveal trabecular meshwork.

**Trabecular meshwork.** The trabecular meshwork is a meshwork which extends from the end of the corneal endothelium to the iris root. Its most superficial layer is formed by the uveal trabecular meshwork, in the shape of roughly cylindrical branches, extending from Schwalbe's ring to the iris root perpendicular to these structures. These trabeculae are anastomosed by similar ele-
Fig. 5. A, Light microscopy of the persistence of pectinate ligament in late-onset congenital glaucoma. The ciliary muscle goes as far as the spur. B, Light microscopy of high insertion of the iris. The ciliary muscle goes as far as the middle of Schlemm's canal.

ments placed horizontally, leaving large intertrabecular spaces between them (Fig. 2, A, B, and C).

The uveal trabecular meshwork is easily identified since in normal eyes it is sparser than the corneoscleral trabecula over which it lies. The uveal meshwork passes over the scleral spur to end at the level of the iris root. These trabeculae are thicker and show more relief than the scleral trabecula. They are seen in gonioscopy as the iris processes described by Busacca, known as the pectinate ligament.

Both terms describe mesodermic remnants in the chamber angle. The uveal meshwork is enveloped by an endothelium in which pores can be seen, continuous with the corneal endothelium.

While the outlines of the corneal endothelial cells and of its nuclei can be barely identified, the size and shape of the trabeculae endothelial cells cannot be appreciated.

No pores were identified in the corneal endothelial cells.

The scleral (or corneoscleral) meshwork can be seen through the spaces of the trabecular meshwork and at a deeper level than the uveal meshwork, through the uveal trabecular spaces. Its trabeculae are parallel to Schwalbe's ring, i.e., perpendicular to the uveal meshwork. These trabeculae are also cylindrical but they are more slender and have smooth outlines, in contrast with the uveal trabeculae, which resemble gnarled tree trunks due to their irregular and wavy outlines. This disposition determines the shape of the intertrabecular spaces of the scleral trabeculum, which are over holes parallel to Schwalbe's
Fig. 6. A to E, Mesodermal tissue: pectinate ligament, late congenital glaucoma. A, The trabecular region shows a dense tissue: uveal meshwork extending from Schwalbe's line (1) to scleral spur (2). These trabeculae are thicker, like gnarled tree trunks. B shows the upper insert of A: between two trabeculae there is a membrane and no intertrabecular space, leaving only small holes. Erythrocytes are retained by this dense meshwork. C shows the lower insert of A—unusual trabeculae. D is taken from the same specimen and shows how the blood cells are retained by this meshwork, compared to E, taken from a normal scleral meshwork where it is possible to see the blood cells going through the intertrabecular spaces freely.
Fig. 6. D and E. For legend see facing page.

ring, with very smooth outlines. These structures are quite different from the intertrabecular spaces of the uveal meshwork, which are larger and quadrangular. Through each of these a number of scleral trabecular spaces can be seen because of their smaller size. Pores can also be identified in the endothelium of the scleral trabeculae. Between the two levels of uveal and scleral meshwork some slender fibers perpendicular to Schwalbe's ring can be seen. These are trabeculae that pass as a bridge over the scleral spur toward Schwalbe's ring, forming the insertion tendon of the longitudinal portion of the ciliary muscle (Brückes' muscle), as has been demonstrated by Dvorak Theobald. This fascicle can be seen in some trabeculectomies as a compact layer that reaches the scleral spur and after veering, gives rise to trabeculae.

Adjacent to the corneal endothelium and above Schwalbe's ring the scleral trabecular endothelium shows a smooth area.

The intertrabecular spaces of the uveal meshwork measure 20 to 30 μ; those of the scleral meshwork are smaller and, since they are oval, show a greater axis 10 μ long and a lesser axis, 5 μ long. Near Schwalbe's ring the greater axis does not change, but the lesser axis is reduced to only 1 or 2 μ, so that the mesh becomes tighter.

**Schlemm's canal.** We have studied Schlemm's canal in two eyes enucleated from 2-year-old children because of a retinoblastoma. The opening of Schlemm's canal was made in triangular blocks with the apex in the center of the cornea including cornea, sclera, and iris. Schlemm's canal was intubated with the inferior blade of Vannas' angled scissors and the section was made holding this blade fixed while sectioning the trabecular meshwork in its upper end, closer to Schwalbe's line, with the other blade. In this way, avoiding pressure on the inside of Schlemm's canal, its integrity can be preserved. This section must be made at the moment when the dehydration with acetone solutions in different concentrations is underway. Figs. 3 and 4 show our findings.

Schlemm's canal has a greater diameter that ranges between 180 and 250 μm. Both this canal and the collectors and aqueous veins are often subdivided by septa, as if there were physiological barriers to the exit of the aqueous humor.

The internal wall of Schlemm's canal shows elongated cells, 75 μm long and 4
μm wide. The nuclei of these cells are spindle shaped, 16 μm long and 4.33 μm wide. Between these cells or on their surface lie minute pores through which the aqueous humor passes. These pores have been carefully described by Bill and Svedbergh1 (Fig. 3, A, B, C, and D).

Fig. 4, A, is a panoramic view of the chamber angle after trabeculectomy. Fig. 4, B and C, are insets showing the areas marked in Fig. 4, A, where the openings of two collectors can be appreciated. These are already subdivided at their origin and their average diameter ranges between 50 and 80 μm.

Congenital glaucoma and late congenital glaucoma. The tissue overlying the normal trabecular meshwork can be referred to by any one of the following synonyms: mesodermal tissue, pectinate ligament, or iridian processes in the trabecular meshwork. This may be either fetal remnants or a developmental anomaly.

In a group of cases of congenital glau-
coma of late onset we observed with the light microscope a dense tissue occluding the sinus, sometimes up to Schlemm's canal and sometimes up to Schwalbe's line, hiding the ciliary band. This is, in fact, an abundant dense uveal meshwork. With Masson's trichrome stain this tissue takes a blue hue.

Another group of cases, diagnosed as pigmentary glaucomas, also presents this pectinate ligament; however under light microscopy and with trichrome stain it is colored pink. It is formed by a net of delicate trabeculae, much smaller than those of the corneoscleral meshwork, not covered by nucleated cells.

These two groups, histologically distinguishable, cannot be identified by gonios-
copy as far as the morphology of the pectinate ligament is concerned.

With light microscopy we can differentiate two main types of anomalies in children with congenital glaucoma: (1) persistence of the pectinate ligament and (2) aplasia of the pectinate ligament.

In the case of persistence, this tissue is formed by dense trabeculae reaching Schwalbe’s ring, associated with an anomaly of the position of the ciliary muscle, its internal surface reaching the level of the middle of Schlemm’s canal.

The same picture may be observed in congenital glaucoma of late onset in adults, but the ciliary muscle is generally in its place, or suffers only slight displacement.

The second form, aplasia of the pectinate ligament, corresponds to what American workers call high insertion of the iris, since the iris root ends at the level of the trabecular meshwork.

The following preparations present an example of this structure. In Fig. 5, A, we can see with light microscopy the persistence of the pectinate ligament. In Fig. 6, A, B, C, D, and E, scanning microscopy of the same specimen shows a dense tissue with predominance of large trabeculae running perpendicular to Schwalbe’s line.

This tissue belongs to the uveal meshwork. Among the trabeculae there is a membrane (endothelium?) leaving relatively small perforated spaces which obstructs the flowing of the erythrocytes.

Fig. 7, A, B, C, and D, shows the scanning microscopy of a specimen of trabeculectomy with trabeculotomy taken from a case of congenital glaucoma in a child 2 months old. The pattern is the same as that seen in congenital glaucoma of late onset, but light microscopy shows a very different pattern (Fig. 5, B). The ciliary muscle goes as far as the middle of the inner wall of Schlemm’s canal.

Pseudoexfoliation of the lens capsule.
In cases of pseudoexfoliation of the lens capsule with glaucoma, the trabecular meshwork presents the same aspect as in simple glaucoma but it appears as if it were dirty, with three types of characteristic elements.

1. The first element can also be observed in other preparations but it is never as abundant as in capsular glaucoma. It consists of pigment granules arising in the posterior face of the iris. We have already described this pigmentation, which exceeds Schwalbe’s line in the lower part of the chamber angle between 3 and 9 o’clock,
Fig. 10. Pseudoexfoliation material in the trabecula in the form of irregular meshwork of spiraling fibrils. (+10,000.)

across 6 o'clock, depositing itself in the posterior face of the cornea.

These granules are small spheres of an average dimension of 0.8 to 1.2 μ; they are also found inside Schlemm's canal.

2. The second element has not been previously described in the trabecular meshwork: it is a typical pseudoexfoliation material made up of an irregular meshwork of fibrils. Its diameter may be measured with high magnification and it oscillates between 600 and 800 A.

3. The third remarkable finding is the clear identification of the cellular membranes of the corneal endothelium. With high magnification, these limits are formed by a wavy membrane jutting out in the anterior chamber. We do not know whether it is the same fibrillar substance or the pigment which has an affinity with the intercellular space. In Figs. 8 to 10 we can observe these findings.

Davanger found this same material, of the same size, on the anterior lens surface and in the posterior chamber region.

Conclusions

A precise microsurgical technique allowed us to identify Schlemm's canal and the trabecular meshwork in 185 out of 190 trabeculectomy specimens. Through correlation of the findings of light and scanning electron microscopy with the clinical study and the gonioscopic image we could confirm or rectify our diagnosis. This method gives new evidence about certain entities whose pathogenesis is still controversial, such as glaucoma associated with pseudoexfoliation of the lens capsule, congenital glaucoma and late-onset congenital glaucoma, pigmentary glaucoma, and cortisonic glaucoma.

REFERENCES
