

Evidence for Posterior Zonular Fiber Attachment on the Anterior Hyaloid Membrane

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PURPOSE. To image the posterior zonular attachment site by using environmental scanning electron microscopy (ESEM).

METHODS. A custom-made device was designed to mount human cadaveric lenses, with the zonule, ciliary body, and sclera attached, inside an environmental scanning electron microscope (ESEM). The mount was designed to allow radial stretching to enhance exposure of the accommodation apparatus. Seven fresh human eye bank eyes (age: 62–82 years; 24–48 hours after death) were dissected, mounted, and imaged in wet mode. The insertion site of the posterior zonule was examined.

RESULTS. Detailed pictures of the accommodation apparatus were obtained in all eyes. A strong relation between the posterior zonule and the anterior hyaloid membrane was observed. Anterior view micrographs showed that posterior zonular fibers originate from the ciliary body and anchor in the hyaloid membrane. From the point of insertion, the fibers continue on their course toward the posterior lens capsule in the plane of the hyaloid membrane.

CONCLUSIONS. Contrary to the classic description, the majority of posterior zonular fibers are not attached directly to the posterior lens capsule, but are anchored to the anterior hyaloid membrane on their path from the ciliary body to the posterior capsule. This finding is in good agreement with several previous observations and models that suggest support of the posterior lens surface during accommodation. (*Invest Ophthalmol Vis Sci.* 2006;47:4708–4713) DOI:10.1167/iavs.06-0441

The architecture of the suspensory ligaments of the lens determines the way forces are transferred to the lens capsule. Consequently, its accurate description is of great importance for understanding and modeling accommodation, and its changes with age leading to presbyopia.

The current descriptions of the zonular architecture are based largely on the extensive anatomic studies by several researchers in the late 1960s and early 1970s,^{1–9} and have been confirmed by more recent studies.^{10–12} These studies led to a description where the zonular architecture is composed of two

main regions, anterior and posterior, and a secondary equatorial region along the canal of Hannover (Fig. 1). The anterior hyaloid membrane is described as a delicate structure in the form of a thin layer that runs from the pars plana to the posterior lens, where it shares its attachment with the posterior zonule via Wieger's ligament,^{13,14} also known as Egger's line.

Experimental evidence suggests a strong relation between the posterior zonular fibers and the anterior hyaloid membrane.¹³ This relation is not accounted for in the classic description of zonular architecture but could have implications for the mechanism of accommodation. However, this relationship has not been fully explored, and remains to be confirmed. Anatomic study of the posterior zonule has been difficult, since it is hidden by the anterior zonule in the front and the anterior hyaloid membrane in the back. In previous anatomic studies, using conventional scanning electron microscopy (SEM), the anterior hyaloid membrane was removed to expose the posterior zonule, thus potentially altering any relationship to it. The goal of the present study was to develop a technique to investigate the relationship between the posterior zonule and the anterior hyaloid membrane, without the removal of the membrane. The technique combines environmental scanning electron microscopy (ESEM) with a custom-made manual lens stretcher for use inside the microscope.

METHODS

An eight segment miniature manual lens stretcher was designed to fit the translation stage of an ESEM (XL-30 ESEM-FEG; Philips, Eindhoven, The Netherlands). The stretcher is designed to conform to the anatomy of a complete human globe and allow dissection and exposure of the posterior chamber with minimal deformation. Seven donor eye bank eyes, ages 62 to 82, were mounted and dissected in the stretcher. The time between death and the experiment ranged from 24 to 48 hours. The eyes were obtained and managed in accordance with the guidelines in the Declaration of Helsinki for research involving human tissue. The complete globe was glued to the stretcher with cyanoacrylate, and the posterior pole, cornea, iris, and excess vitreous were removed to expose the accommodation apparatus (Fig. 2). Special care was taken during vitreous dissection to avoid damage to the hyaloid membrane. The sclera was then cut between each of the eight segments and stretched 4 mm in diameter to maximize the exposure of posterior structures. The stretch is mostly taken up by the ciliary body. On average, the ciliary ring stretches 0.5 mm in diameter and the lens 0.2 mm. The goal of stretching is to provide enough space between the ciliary body and the lens to allow posterior structures to be seen through the anterior zonule without significant damage to the sample. The tissue mounted in the stretcher was slightly fixed in 2% formaldehyde solution in the stretched state for preservation purposes to avoid decomposition. Fixation was required because in most cases the ESEM was not available immediately after preparation. The time between preparation and imaging ranged from 4 to 48 hours.

Samples were scanned anteriorly and in cross section in environmental gaseous secondary electron GSE detection mode, using water vapor gas. The ESEM parameters (beam energy, contrast, brightness, working distance, and pressure) were adjusted for each eye, by relying

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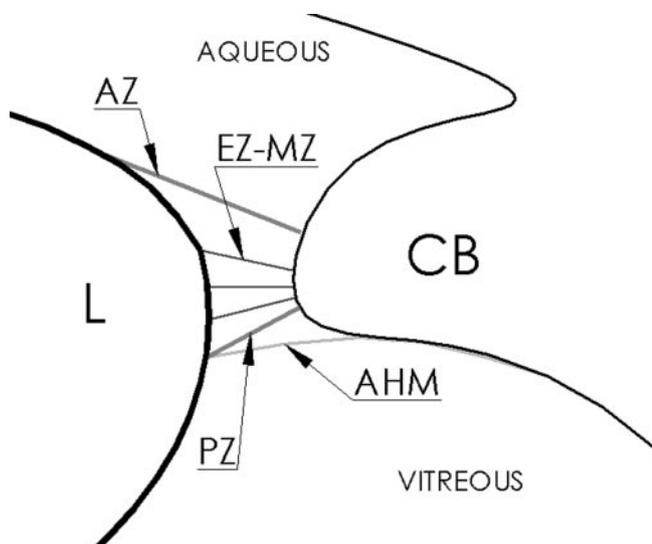


FIGURE 1. Classic architecture of the accommodation apparatus. L, lens; CB, ciliary body; AZ, anterior zonule; EZ-MZ, equatorial and meridional zonule; PZ, posterior zonule; AHM, anterior hyaloid membrane.

on feedback information from the live images, until the best image was obtained. During image acquisition, the sample gradually dehydrates due to the partial vacuum (1-5 Torr) inside the ESEM chamber. The dehydration process has two distinct progressive steps. In the first step (initial dehydration, ~20 minutes), excess moisture remaining from the immersion medium evaporates from the sample's surface until anatomic features become visible. This marks the beginning of the second step (hydration window), which (~1 hour) starts once the surface moisture has evaporated and the tissue bulk starts to dehydrate progressively. A slow progressive shrinking of the ciliary body occurs, but changes are initially very slow and are not detected during the image-acquisition scan (~1 minute). At the end of the hydration win-

dow, the anatomic changes due to dehydration occur at a faster rate and affect image acquisition if they occur during the photograph scan. The zonular fibers start to rupture, and tears can develop in the hyaloid membrane. In addition, as dehydration progresses, tissue may start accumulating charge, which compromises image contrast. Images taken during a typical dehydration sequence are shown in Figure 3.

Since the effects of dehydration are progressive, there was no clear objective endpoint to the image collection. Most of the micrographs showing the posterior zonular insertion into the hyaloid membrane were recorded in the slow-dehydration phase of the hydration window, before structural damage to the zonules or hyaloid membrane. However, images were acquired even after the onset of zonular fiber rupture or hyaloid membrane tears, because some of these images provide valuable qualitative information, despite tissue damage. The imaging session was generally stopped only after no useful information could be obtained from the images, either due to excessive tissue damage or to loss of contrast.

RESULTS

Detailed pictures of the accommodation apparatus were obtained in all eyes. The stretch provided by the lens stretcher exposed the structures and provided a clear, unobstructed anterior view from the anterior zonule to the hyaloid membrane. The lens; zonular lamella; anterior, posterior, and meridional zonular fibers; ciliary body; and hyaloid membrane could be identified with ease. The large depth of field (>2 mm) obtained at magnifications of 50× or less allows the different zonular fiber layers and the hyaloid membrane to be in focus in a single image.

Images show that the posterior zonule interacts with the anterior hyaloid membrane. In none of the anterior views were zonular fibers found to go from the ciliary body directly to the posterior lens (Fig. 4). Instead, the fibers projected into the anterior hyaloid membrane. Zonular fibers appear to bond with the membrane at the point of contact and from there continue their course toward the lens along the same plane (Fig. 5). A

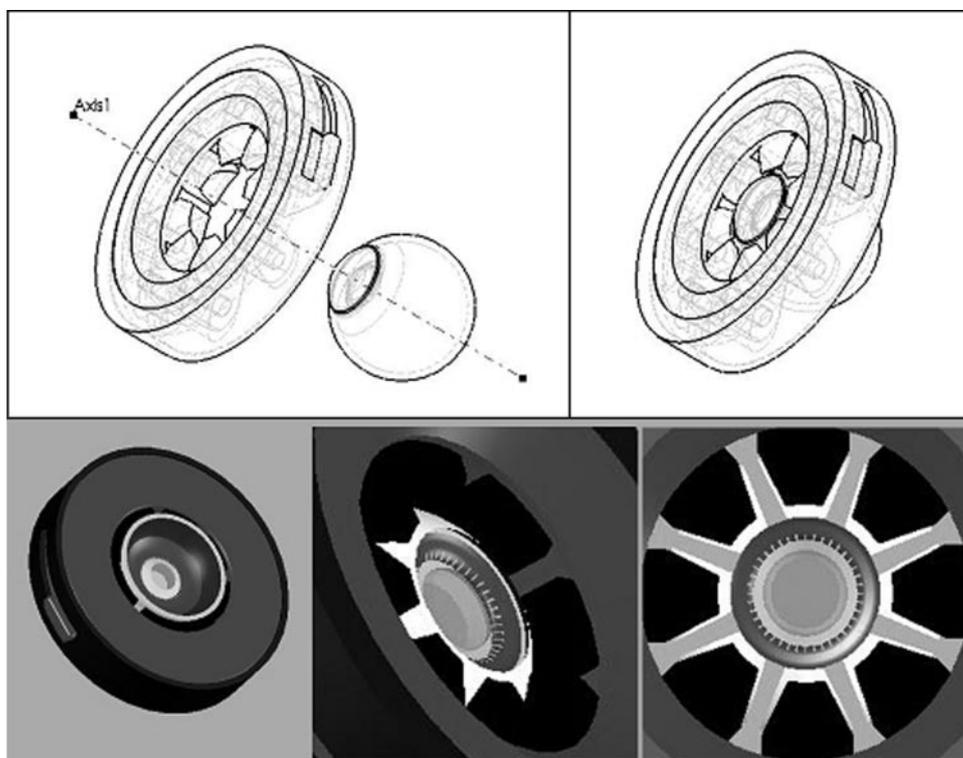


FIGURE 2. Tissue dissection procedure. *Top left:* eye is aligned with lens stretcher along the optical axis (Axis 1). *Top right:* the cornea is centered within the aperture and the sclera is glued to the lens stretcher segments. *Bottom left:* the posterior pole is dissected, and excess vitreous is removed. *Bottom center:* the cornea and iris are dissected. *Bottom right:* the sclera is cut between segments to allow stretching.

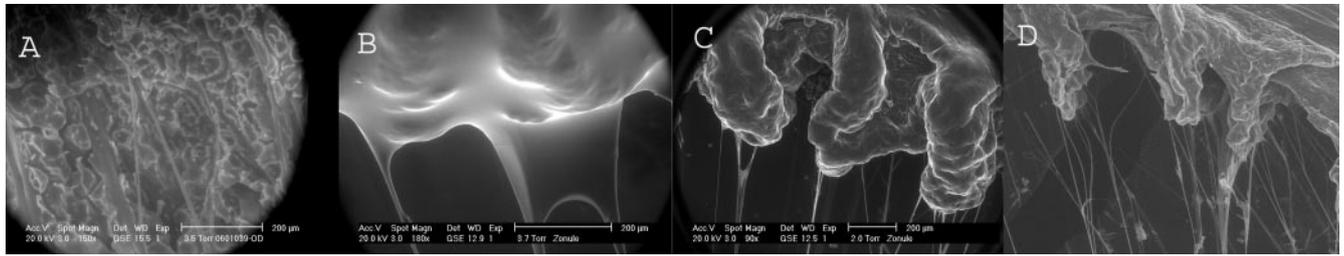


FIGURE 3. Tissue hydration stages during ESEM. (A) Initial hydrated stage of the ciliary body, with no visible features. (B) Ciliary body with surface moisture during the dehydration process. (C) The ciliary body as seen during the hydration window. (D) Terminal dehydration, showing permanent tissue damage and deformation.

detailed view of the insertion site revealed a tissue matrix that may serve as the attachment mechanism (Fig. 5). Cross-sectional images showed some zonular fibers inserting into the hyaloid membrane at points that are very close to the lens (arguably on Wieger's ligament). However, nearly all of the zonules insert into the hyaloid membrane proximal to the ciliary body (Fig. 6), confirming the anterior view observations.

Some images were acquired in samples where the tissue ruptured under dehydration stress in the ESEM chamber. Although the zonular fibers and the hyaloid membrane showed critical signs of damage, posterior zonular fiber insertions on the anterior hyaloid membrane were observed to be preserved. All the posterior zonules remained attached (or embedded) in the membrane. This observation suggests that the posterior

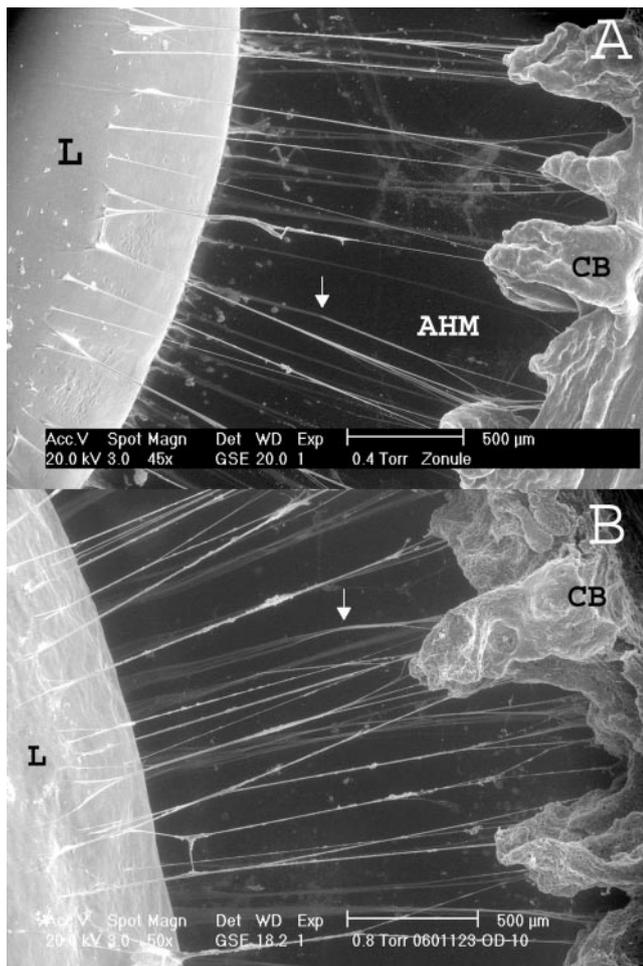


FIGURE 4. Anterior view of the accommodation apparatus in two different subjects (A, B). The path of the anterior zonular fibers is clearly visible, running from the ciliary body (CB) to the lens (L) in a straight line. Posterior zonules appear as diffuse lines in the background; they lie on the plane of the hyaloid membrane. Their origin in the ciliary body is hard to identify in this view. *Arrows:* examples of zonular fibers that run from the ciliary body to the hyaloid membrane, before continuing to the lens. No posterior fibers were found to traverse directly from the ciliary processes to the posterior lens.

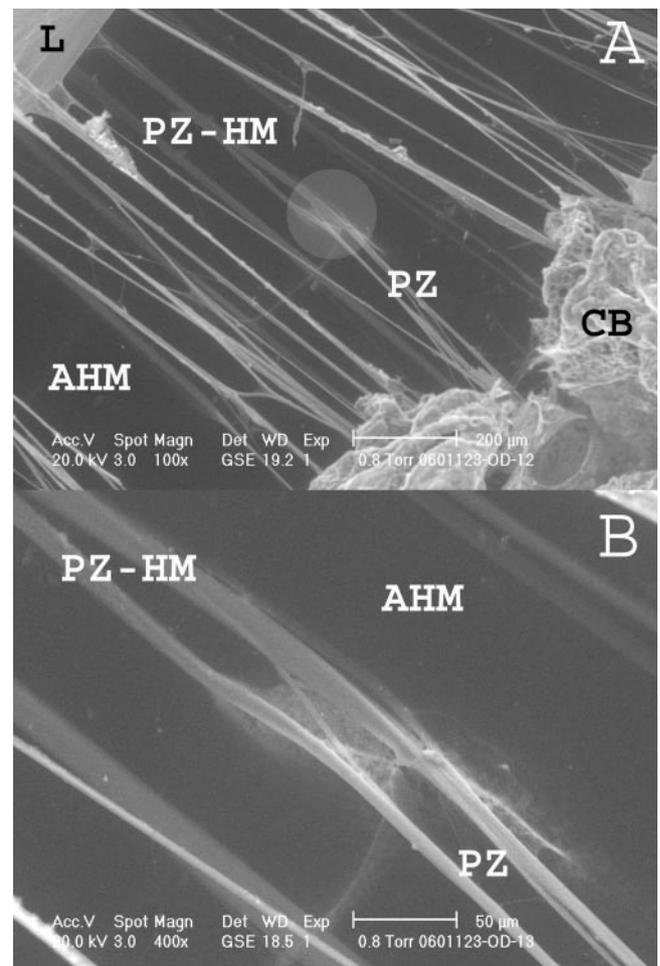


FIGURE 5. Detail of posterior zonular fiber inserting in hyaloid membrane. (A) A posterior zonular fiber (PZ) originating from the ciliary body (CB) anchors in the anterior hyaloid membrane (AHM). It continues its course toward the posterior lens, embedded in the membrane (PZ-HM). (B) Detail of the insertion point of a posterior zonular fiber in the hyaloid membrane. A tissue matrix can be observed at the interface. The fiber can be seen after the insertion, in the plane of the membrane.

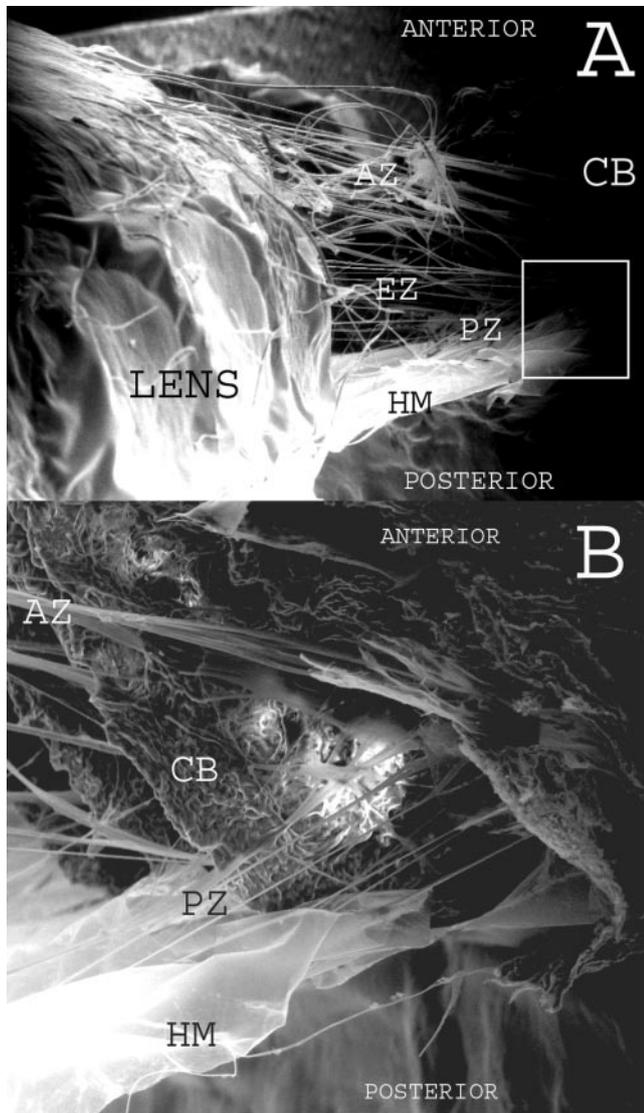


FIGURE 6. Cross-sectional view of zonular architecture. (A) Abundant anterior (AZ) and equatorial (EZ) fibers are seen running from the ciliary body (CB) to the lens periphery. A few fibers are visible with a posterior orientation (PZ). The hyaloid membrane is visible as a white sheath at the bottom (HM). A detailed view of the boxed area is seen in (B). (B) A closer look toward the ciliary body reveals numerous posterior zonules projecting into the anterior hyaloid membrane very close to their origin in the ciliary body. The proximity of the insertions to the ciliary processes makes them invisible in the anterior views.

zonules are strongly connected to the anterior hyaloid membrane (Fig. 7).

DISCUSSION

Our results provide strong evidence that, contrary to the classic description, the posterior zonule does not insert predominantly into the posterior lens capsule. The majority of the posterior zonular fibers were found to insert into the anterior hyaloid membrane on their path toward Wieger’s ligament on the posterior lens capsule (Fig. 8).

The conventional SEM methods used in studies that led to the classic description of the zonular architecture had technical limitations that may have interfered with the observation of this feature. Conventional topographic SEM study of the human zonule typically requires separate scanning of its anterior

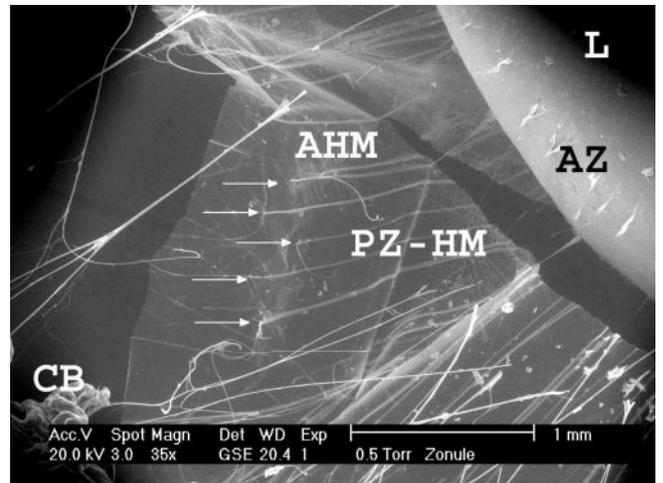


FIGURE 7. Anterior view of circumlental space showing tissue failure due to dehydration stresses. Ruptures in the anterior hyaloid membrane (AHM) and anterior zonular fibers (AZ) are evident. Arrows: points of insertion of posterior zonular fibers in the membrane. Posterior zonular fibers remain embedded in the hyaloid membrane (PZ-HM). L, lens; CB, ciliary body.

and posterior fibers. After the anterior fibers are imaged, the tissue has to be manipulated to expose the posterior fibers to the electron beam. Because the anterior hyaloid membrane covers the accommodation apparatus posteriorly, it was usually removed to expose the posterior zonule for imaging.^{4,5,11} In other studies, the lens was extracted by cutting the zonules and anterior vitreous to preserve the zonular insertion in the lens capsule³ for examination. These preparation techniques reduced the chances of observing any relation of the posterior zonule with the anterior hyaloid membrane. In previous studies made by our group with conventional SEM, dehydration was identified as the most deleterious step of tissue processing. Delicate structures such as the lens capsule, zonular fibers, and hyaloid membrane sustained damage that affected their proper anatomic exploration (Lamar et al. *IOVS* 2005;46:ARVO E-Abstract 737).

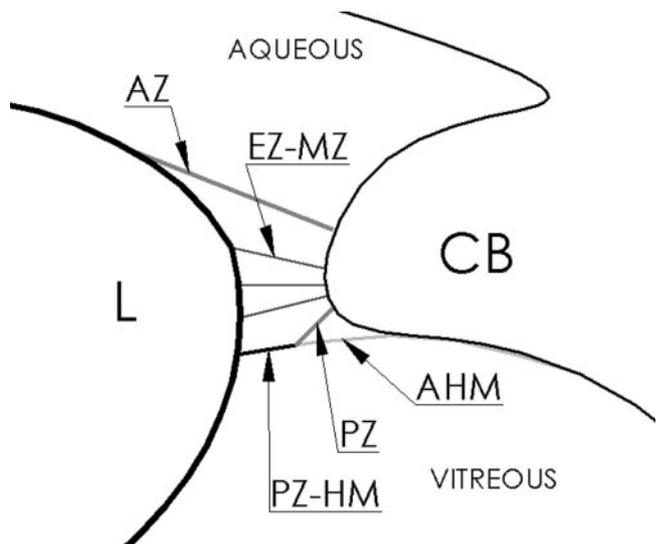


FIGURE 8. Proposed architecture of the accommodation apparatus. AZ, anterior zonule; EZ-MZ, equatorial and meridional zonule; PZ, posterior zonule; AHM, anterior hyaloid membrane; PZ-HM, posterior zonule embedded in the hyaloid membrane.

ESEM provided the means to study the zonule and its geometrical organization thoroughly, with detail comparable to conventional SEM. The main advantage of ESEM is that it allows “wet-mode” imaging under low vacuum. Organic solvent dehydration and critical-point drying used in conventional electron microscopy preparation are not necessary. The detection mechanism in the ESEM also avoids the need for conductive coating of the specimen. These two features reduce the risk of alteration of anatomic structures during preparation. The large depth of field of the ESEM, combined with the added anatomic exposure gained with the stretcher, enabled us to avoid removing the hyaloid membrane for imaging. With this technique, structures at different depths from the anterior zonule to the hyaloid membrane could be seen in focus in a single image. This allowed us to explore the posterior zonule from an anterior view, through the gaps between the anterior zonular fibers. This eliminates the need of peeling or removing the hyaloid membrane to explore the posterior zonule from a posterior view. Since the hyaloid membrane was left intact, with remnants of vitreous attached, no images were taken from a posterior view.

Although ESEM has the capability of imaging unfixed samples, the limited availability of fresh tissue and scheduling delays between preparation and imaging made this approach impractical. Most of the samples were fixed for preservation purposes, using 2% formaldehyde solution. Unfixed samples were scanned to determine whether the posterior fiber adhesion to the hyaloid membrane could be an artifact caused by fixation. The results showed no macroscopic structural difference between unfixed and fixed tissue. Posterior zonular insertions in the hyaloid membrane and zonular fibers traveling through the hyaloid membrane were found in the unfixed sample (Fig. 9). However, the integrity of the sample and the optimal hydration window duration were significantly reduced in the unfixed tissue.

Our findings are consistent with several previous anatomic observations. As early as 1942, through gross dissection, Minsky¹⁵ described a “hyalo-zonular leaf”, consisting of zonular fibers that run on the anterior surface of the hyaloid membrane. Later, Davanger⁶ and Reich et al.¹⁶ showed SEM micrographs of zonular fibers embedded in the hyaloid membrane. An ingenious study by Albrecht and Eisner,¹⁷ involving zonular stretching, showed further evidence of a complex system of posterior fibers. They describe a set of fibers running from the hyaloid membrane to the posterior lens capsule, which they named the “hyalo-capsular zonule,” and another set from the hyaloid membrane to the ciliary body, which they named the “hyalo-ciliary zonule.”

In 1978 Streeten and Pulasky¹³ convincingly demonstrated a relationship between the anterior hyaloid membrane and the posterior zonule. They observed that during cataract extraction, remnants of the posterior zonular fibers were rarely found on the lens, whereas remains of the anterior, equatorial, and meridional fibers were present. Posterior fibers were found to be left behind, still adherent to the anterior hyaloid membrane, which showed that the relationship was not an artifact or inconsequential, but that it was authentic and significantly stronger than that of the zonule to the lens itself. When Streeten and Pulasky attempted to explore the relationship between the posterior zonule and hyaloid membrane with electron microscopy, they encountered many difficulties related to tissue handling and processing, including rupture of fibrillar attachments and hyaloid membrane peeling. They specifically discuss that the zonular fibers become more adherent to the lens after fixation, which could help explain why previous investigators were able to see the posterior fibers, even after removal of the hyaloid membrane. In addition, we have observed that in dehydrated preparations, the thin hyaloid

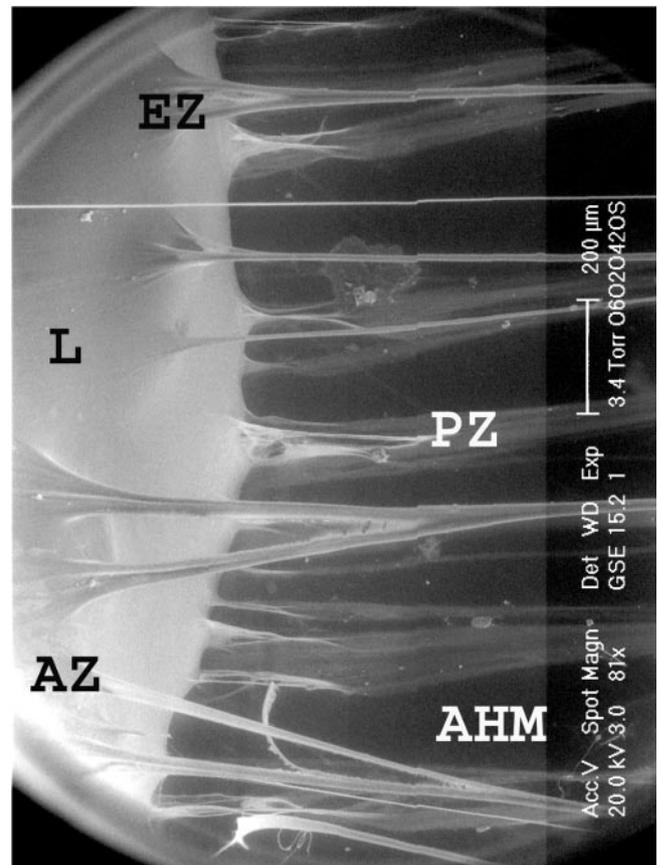


FIGURE 9. Anterior view of zonular insertion in an unfixed sample. The anterior (AZ) and equatorial (EZ) zonular insertions are seen. Posterior zonules (PZ) are seen running through the hyaloid membrane (AHM) before their insertion into the lens (L). Dehydration rate, tissue damage, and charging effects were increased in unfixed samples, affecting image quality and stability.

membrane becomes weak and brittle and can be easily separated from the lens and zonules, leaving behind the more resistant posterior zonular fibers. Davanger⁶ and Rohen⁴ have been able to observe the posterior zonule, but specifically mention the removal of the hyaloid membrane after fixing and dehydration in their methods. A more recent study by Canals et al.¹¹ is able to show micrographs of the posterior zonule after removal of the hyaloid membrane, but again, the group specifically points out that the intense adhesion of the hyaloid membrane to the posterior zonules leads to observations of remnants of hyaloid membrane on the posterior zonular fibers.

The posterior zonular attachment to the anterior hyaloid membrane could have an effect on the mechanics of accommodation. Because anterior zonules run a straight path from the ciliary body to the lens, any contraction or relaxation of the ciliary body directly reflects on the anterior lens capsule. In contrast, the force transmitted from the ciliary body to the posterior lens, via the posterior zonular fibers, will be mediated by the hyaloid membrane. The forces acting on the posterior lens capsule could effectively be less intense than those exerted on the anterior capsule by the anterior zonule. This is consistent with observations of lens behavior during accommodation.^{18–24} The changes in posterior lens curvature, as well as the displacement of the posterior pole during accommodation, have been reported to be much less than the changes on the anterior lens. Our findings provide anatomic evidence that corroborate to some extent models that advocate

posterior lens suspension or support, like those of Coleman^{25,26} or Koretz and Handelman²⁷ and Strenk et al.²⁸

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