The zonular insertion: a scanning electron microscopic study

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The zonular insertion on the human lens capsule has been studied with scanning electron microscopy. The whole insertional area is covered by a variety of 35 to 55 nm fibrils, including those of the major inserting zonules, the meridional zonules, capsular surface fibrils, and a nonoriented fibrillar layer over the zonules. All appear to be zonular in type. The meridional zonules span the equator and are a form of insertion shared by the three levels of major zonules, probably helping to distribute their lateral pull. Surface rippling over the zonular insertion appears to result from greater contraction of the oriented fibers than of the non-oriented fibrils and superficial capsule. Surface meshwork fibrils attach to the capsule by becoming embedded in a granular capsular matrix 0.5 to 1 μ thick.

Key words: zonule of the lens, scanning electron microscopy, zonular insertion, accommodation, zonular lamella.

The mechanism of zonular insertion on the human lens capsule has received only sporadic attention in the modern era, although it is essential to an understanding of both normal accommodative function and pathology involving these structures. An enduring concept of zonular attachment has been that of a "zonular lamella" or special layer on the lens surface into which the zonules insert, as described by Berger in 1882. By transmission electron microscopy (TEM) the zonular lamella of light microscopy resolves into two layers:
Fig. 1. Equator of lens. Zonular insertional area appears light against dark lens capsule background. Three levels of zonular insertion are seen: anterior (A), equatorial (E), and posterior (P). Tearing away of anterior hyaloid membrane has left posterior zonular insertion ragged.

(Scanning electron micrograph, x64.)

(1) the loose superficial capsule surrounding the lens and (2) an outer layer of inserting zonules in the equatorial region. It is not clear whether the zonular portion is a continuous layer or intermittent, appearing only where the zonules directly attach. Since the number of zonules is said to be considerably reduced in aging, with those at the equator being especially sparse or absent, an intermittent covering might be expected in the adult.

Scanning electron microscopy (SEM) should be ideal for studying these problems of zonular fiber distribution, since the zonule is essentially a surface tissue. However, reports of the zonular insertion by SEM have been fragmentary in both man and animals. Technical problems with preservation of the fragile zonule and drying of the dense lens material, coupled with low resolution, decrease the value of some of the early descriptions. In only a few studies has resolution been sufficiently high to visualize the individual fibrils of the normal zonule. The presence of a zonular lamella into which

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the inserting zonules "disappear" has generally been accepted but not defined. The ultimate attachment of the zonules to the lens is known to involve penetration of zonular fibers into the superficial lens capsule, but may also include some type of physicochemical binding commonly referred to as "glue." With one exception the SEM studies have not thrown light on this problem. Recently Farnsworth and co-workers have described a fibrillar ridging of the anterior lens surface which they suggest may serve as zonular attachment sites of the Velcro type. The surface fibrils on these ridges were considered by the authors to be capsular in origin, although they were of the same 50 to 70 nm. diameter as the fibrils of the zonule.

The present study will describe all areas of the zonular insertion as seen by SEM, with special consideration of the zonular lamella and capsular attachments.

Methods and materials

Thirty human lenses were examined with SEM. Six were lenses removed at cataract surgery and fixed immediately in 2.5 percent glutaraldehyde. Twenty-four lenses were obtained from Eye Bank eyes, 4 to 12 hours after death. These lenses were prepared in a variety of ways, including in situ fixation and extraction while unfixed, with the use of a capsule forceps. Most lenses were removed before fixation by cutting the zonules and anterior vitreous close to the ciliary processes under the dissecting microscope, to preserve the maximum number of zonules on the lens capsule. Eight lenses were fixed in 2.5 percent glutaraldehyde and the remainder in 10 per cent neutral buffered formalin. Most of the specimens were postfixed in osmium, dehydrated in graded acetones, and dried in a critical-point drier. They were then coated with carbon and gold-palladium and examined in an ETEC or a Coates and Welter scanning electron microscope. Eight lenses were postfixed in osmium-thiocarbohydrazide (TCH) and coated only with gold-palladium.

Results

When a lens is extracted with its attached zonules the entire width of the zonular insertion can be viewed in the scanning electron microscope (Fig. 1). This insertional region appears as a well-demarcated light area against a darker lens capsule background. The attached an-
Fig. 3. Final termination of smallest anterior zonular bundles (Z). Fibrils partially obscured by interfibrillar material as they join surface fibrillar meshwork. (Scanning electron micrograph, ×21,000.)

Fig. 4. Anterior lens capsule. A, 1 mm. in front of zonular insertion showing mixture of random fibrils and pebbled surface. (Scanning electron micrograph, ×16,800.) B, Center of lens with pebbled texture. (Scanning electron micrograph, ×16,800.)
The anterior, equatorial, and posterior zonules join this band at fairly regular intervals, often in double rows.

The anterior and posterior zonules insert as bundles of 25 to 60 μm diameter in their contracted state. The equatorial bundles can be as large (Fig. 1) but are often small, from 10 to 15 μm in diameter, seen at all ages into the ninth decade. As the zonules spread out on the capsule the larger ones rapidly separate into 5 to 10 μ bundles. The anterior zonules continue to intermingle and decrease in size over a 0.3 to 0.4 mm flat insertional area (Fig. 2, A), (Linear measurements are not accurate due to a 20 percent shrinkage of the lens in processing.) An interfibrillar material often obscures the fibrils of the larger zonules, but where visible they are 35 to 55 nm in diameter. Loose peripheral fibrils blend into a fibrillar meshwork on the capsular surface (Fig. 2, B). This meshwork is thrown into consistent but irregular ridges in the inserting area. The anterior zonules end as 0.3 to 0.4 μm fanning bundles, blending into the surface layer in basketweave fashion (Fig. 3).

The fibrils on the capsular surface are
also 35 to 55 nm in diameter. They continue for 0.2 to 0.3 mm. beyond the apparent ends of the zonules. Random fibrils may be seen for another 0.4 to 0.6 mm. centrally (Fig. 4, A). The typical pebbly surface shown by basement membranes is increasingly evident among these surface fibrils (Fig. 3). In the central 3 to 4 mm. of capsule pebbly granules form the only textural element (Fig. 4, B). They range in size from 40 to 160 nm., with the larger ones more common near the zonules. This characteristic granularity of bare lens capsule is useful in identifying areas of zonular loss or admixture of zonule and capsular material.

Patches in which the fibrillar layer has been abraded are helpful in elucidating the nature of this layer (Fig. 5). In the anterior insertional area it is about 0.5 to 1 \( \mu \) thick, with a tendency to contract, roll outward, and be thrown into ripples. Where the base of the fibrillar layer is exposed the attachment of its fibrils to a deep meshwork of fibrils can be appreciated (Fig. 5B). An admixture of interfibrillar matrix and capsular granules covers them. Some radially oriented longer fibers of 0.3 to 0.7 \( \mu \) width apparently travel in this layer also (Fig. 6).

Between the anterior and posterior zonular insertions the fibrillar layer is thickened by the addition of large numbers of meridional fibers of 3 to 7 \( \mu \) in width, causing striations longitudinally across the whole equator (Fig. 7). These are contributed to by vertical arms from all three levels of zonules. Horizontal rippling across this whole layer of zonules can be marked (Fig. 7, B). Over the surface here as in most other areas there is a loose meshwork of fibrils the same size as the zonular fibrils, bridging the spaces between them (Fig. 7, D). When abraded parallel to the
Fig. 6. Frayed patch in fibrillar surface layer showing longer aggregated fibrils among the short fibrils of the meshwork. (Scanning electron micrograph, x21,500.)

oriented zonules these meshwork fibrils are shown adhering to the capsule superficially (Fig. 8). This perilzonal meshwork appears to blend freely with the straight zonular fibrils so that no significant difference is seen between them. Deep zonular penetration is seen occasionally in abraded areas (Fig. 9).

The equatorial and posterior zonules insert more vertically than the anterior zonules. Their bundles are lost sight of quickly as they disappear into the even thicker fibrillar layer present here. The posterior zonules have a fanning insertion like the anterior, but more obscured by meshwork fibrils (Fig. 10). The insertion is 0.4 to 0.5 mm. in width, ending in a jagged border when the anterior hyaloid membrane is torn away (Fig. 1), which is the usual occurrence in the adult. Occasional segmentally contracted zonules are seen here and in the anterior insertion (Fig. 11).

Discussion
By SEM the whole insertional region is covered by either aggregated zonular hun-
Fig. 7. Meridional zonules (MZ's). A, MZ's producing longitudinal striations on lens equator. Anterior zonules at top, posterior below. (Scanning electron micrograph, ×195.) B, Horizontal rippling across elevated MZ's. (Scanning electron micrograph, ×2,000.) C, Equatorial zonule joining meridional rows. (Scanning electron micrograph, ×470.) D, Fibrillar meshwork over whole meridional zonular layer. (Scanning electron micrograph, ×26,500.)
Fig. 8. Pre-equatorial zonule inserting into meridional layer. Abraded edge shows layer to be 1 to 2 µ thick. Fibrils adherent to bare lens capsule, lower right. Zonular fibrils merge with those of fibrillar surface meshwork. Straight fibrils of meridional zonules visible through the meshwork (arrows). (Scanning electron micrograph, ×6,300.)

dies or fibrils. Thus the outer or zonular portion of the zonular lamella appears to be a continuous layer, although undoubtedly very thin in some interzonular areas. The question is whether all of the fibrils seen on the lens in SEM are zonular, since their degree of organization is so different. Besides the obvious inserting and meridional zonular fibrils there are the less aggregated capsular fibrils and the loose peri-

zonular fibrils. Morphologically there is no significant difference among them with SEM, since they all measure 35 to 55 nm. in diameter. This is four to five times the size of zonular fibrils we and others have measured in TEM, possibly representing coating thickness, and is within the range described by other workers with SEM. The suggestion that the capsular surface fibrils are part of the normal capsular
fibrillar structure does not seem likely, since they do not appear in areas of bare or abraded capsule, and are too large. With TEM the fibrils of the normal lens capsule (as distinct from aging inclusions) in our material are 1 to 3 nm. in diameter, whereas zonular fibrils average 10 nm.

The possibility that the perizonular fibrils might be vitreous is bound up with the question of whether the whole zonule is a modified vitreous. TEM should demonstrate whether they have the lucent core of a typical zonular fiber but ultimately biochemistry must answer the question of relationship.

Most of the insertional area is covered by meridional zonular fibers. The striations which these cause on the lens equator have been pictured before in SEM but their relation to the large inserting zonules has not been fully appreciated. While they are clearly contributed to by the three levels of zonules, there is some suggestion that their bulk is greater than can be accounted for by the inserting zonules. Equatorial zonules are indeed sparse and do not contribute much to this pattern. Functionally the orientation of the meridional zonules and their complete coverage of the equator would seem designed to spread the lateral pull of the major zonules more smoothly over the equator, and perhaps to accomplish an equatorial squeeze when the major zonules are relaxed in accommodation.

Concentric rippling of the superficial
Fig. 10. Insertion of posterior zonules on left. Anterior hyaloid membrane (AH) is peeling back on the right. (Scanning electron micrograph, ×210.)

Fig. 11. Posterior zonules folded back on themselves showing smaller fibers entering fibrillar meshwork. One zonule is segmented, also seen occasionally in other areas. (Scanning electron micrograph, ×1,075.)
capsule in the insertional area, as noted by Farnsworth and co-workers, is a consistent feature in several species with different fixatives and is seen in many published TEM figures, so it must represent a basic characteristic. It is most marked where the major zonular attachments are loose or absent, so the Velcro-type adhesive function suggested by these authors seems questionable. Velcro consists of two different opposing surfaces. The loose capsular fibrils bear a superficial semblance to the loops of one Velcro surface, but there is no apparent counterpart to the stiff hooks of the necessary opposing surface.

The variability of the concentric rippling suggests that it is a plastic feature, related to the malleability of the loose superficial capsule and the surface fibrillar meshwork when subjected to the contraction or elastic tension of the oriented zonules. Small hidden zonular bundles probably also contribute to this anteroposterior tension. Rippling of the fibrillar meshwork over the meridional zonules clearly results from greater contraction of the oriented zonular fibrils than the unoriented.

Not much can be said of the deep zonular insertions with SEM. The attachments which were seen consisted of individual fibrils blending with the surface meshwork or capsule. The surface meshwork fibrils were embedded to a depth of 0.5 to 1 μ in capsular matrix, as judged by the admixture of pebbly granules and gluelike material.

REFERENCES

(End of Symposium—Part I)