Scanning Electron Microscopy of the Auditory Teeth along the Mouse Cochlear Duct

Babür Küçük1,2 and Kazuhiro Abe1

Department of Anatomy1 and Department of Otolaryngology2, Hokkaido University School of Medicine, Sapporo, Japan

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Summary. The auditory teeth in the spiral limbus of the cochlear duct are located under the limbal portion of the tectorial membrane and separated by furrows lodging the interdental cells. In this study, the shape, arrangement and distribution of the auditory teeth in the cochlear duct of adult mice were examined by scanning electron microscopy after removing the tectorial membrane and the interdental cells with chemical maceration methods.

The auditory teeth appeared on the top face of the spiral limbus between the edge of the vestibular lip and the Reissner membrane. The teeth on the vestibular lip side possessed elongated upper plates and formed a continuous row resembling the keyboard of a piano; the teeth were separated by radially oriented parallel slits. The elongated teeth decreased in length from the base to the apex of the cochlear duct. The teeth on the Reissner membrane side showed star-shaped upper plates separated by slits and gaps. The population density of the star-shaped teeth decreased from the base to the apex, widening the gaps to hold the interdental cells. The upper plates of the teeth occupied about 75% of an extent of the tooth zone in the hook and first basal half turn, and about 55% in the apical turn.

The regional differences of the auditory teeth are considered to be closely related to local functions of the tectorial membrane and the interdental cells.

In the cochlear duct the auditory teeth are connective tissue protrusions of the spiral limbus located on the primary osseous spiral lamina and lying under the tectorial membrane attached to the spiral limbus. The teeth are separated by furrows filled with the interdental cells. The auditory teeth have long been known by light microscopy (Retzius, 1884; review by Ebner, 1902; Fieandt and Saxén, 1936; Borghesan, 1969) and more recently by transmission electron microscopy (Iurato, 1962; Ilberg, 1968; Thorn et al., 1979), but the morphology of the auditory teeth has not been considered in relation to the hearing function of the cochlea.

Iurato (1962) demonstrated by polarized light microscopy that the teeth comprised densely packed connective tissue fibers. These fibers are inserted into the primary osseous spiral lamina, suggesting that the teeth support the tectorial membrane and the interdental cells (Küçük and Abe, 1989). The tectorial membrane is believed to be secreted by the interdental cells (Iurato, 1962; Voldrich, 1967; Ishiyama et al., 1970; Lim, 1970; Mira, 1971; Arnold and Vosteon, 1973). It is well known that the tectorial membrane vibrates together with the basilar membrane with sound waves, stimulating the sensory cells in the organ of Corti. The tectorial membrane varies in size and vibratory pattern from the base to the apex of the cochlear duct (Steel, 1983; Streltsoff et al., 1985), implying that the anatomical organization of the auditory teeth varies along the cochlear duct in relation to regional functions of the interdental cells and tectorial membrane. In order to clarify this point, we examined the three-dimensional morphology of the auditory teeth along the entire course of the cochlear duct by scanning electron microscopy (SEM).

The auditory teeth can be exposed for direct observation by SEM after removing the tectorial membrane and the epithelial cells of the cochlear duct by a chemical maceration method (Low and McClugage, 1984). This study represents the first three-dimensional SEM visualization of the shape and arrangement of the auditory teeth in the cochlear duct.
MATERIALS AND METHODS

In this study, 24 cochleae from 12 dd-mice of both sexes at 3–6 months of age were used.

Light microscopy

Six temporal bones from 3 mice were fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2 h, decalcified in 5% EDTA overnight, postfixed in 2% OsO₄ for 2 h, dehydrated in ethanol and embedded in Epoxy resin. Sections of cochlea were then cut at 1 μm in thickness across or along the modiolus, stained with toluidine blue and observed with a light microscope.

Scanning electron microscopy

Exposure of the auditory teeth

Fourteen temporal bones from nine mice were immersed in 1% H₃BO₃ solution for 12 h, and the cochlear modiolus was exposed by removing the outer wall of the cochlea using needles under a stereomicroscope. To remove the cochlear duct epithelium and expose the auditory teeth, the specimens were then placed in 2% OsO₄ for 48 h, dehydrated in alcohol, ultrasonicated for 2–3 min, and transferred into isoamyl acetate. After critical point drying, the specimens were attached onto aluminium stubs, coated with platinum-palladium with an ion coater and observed with a SEM.

Styrene resin cracking of the cochlea

Four cochleae were embedded in styrene resin (TANAKA et al., 1974) after removing the epithelium of the cochlear duct as described above. After polymerization of the resin, the specimens were cracked with razor blades in various directions, immersed in propylene oxide to remove the resin, transferred to isoamyl acetate, treated as above and observed with a SEM.

Quantitative observations

In the top view images of the cochlea, the width of the area distributed with the auditory teeth and the width of the tympanic lip area between the habenular openings and the edge of the vestibular lip were measured at points dividing the hook and every half turn of the duct into 4 arcs. The width of the spiral limbus was obtained as the sum of the widths of the above two areas.

The length of the auditory teeth, relationships between the upper surface of the teeth and interdental spaces between the teeth were determined for each 140 μm-long segment of the hook and every half turn. The proportion between the upper surface of the teeth and the interdental spaces between the teeth was obtained by a point counting method as follows.

Fig. 1. Light micrographs showing the spiral limbus of the cochlear duct. AT auditory teeth, IDC interdental cells, RM Reissner membrane, TM tectorial membrane, VL vestibular lip, TL tympanic lip, ISC inner sulcus cells, C Corti’s organ, BM basilar membrane. Toluidine blue. a. Cross section of the spiral limbus (center). ×300. b. Section of the vestibular lip on a plane parallel to its top surface. The upper direction is to the inner sulcus side and the lower to the Reissner membrane side. ×800
On the micrographs at 1,000X of magnification, 30 points of regular interval were plotted both on the inner and outer margins of the 140 μm-long tooth zone, and each line binding two points of a pair on the inner and outer margins was regularly divided by 20 points; from the number of the points hitting the teeth or interdental spaces in each row of the points between the outer and inner margins of the zone, the proportional area of the teeth and interdental spaces occupying each address was calculated. From the total number of points hitting the teeth and spaces, the proportion of the area occupied by the teeth or spaces in each regional tooth zone was determined.

For quantitative analysis, 4 cochleae were used.

**RESULTS**

**Light microscopy**

Under the inner half of the tectorial membrane, the auditory teeth form the upper convex surface and vestibular lip of the spiral limbus, which is elevated like a billow from the tympanic lip, of the limbus across the inner spiral sulcus covered with a layer of inner sulcus cells (Fig. 1a). The teeth are columnar in shape, showing plate-like expansions at the top face (Fig. 1a). Between the teeth were found round or oval interdental cells with light cytoplasm (Fig. 1). The cell bodies of the interdental cells were situated under the upper plates of the teeth on the side of the vestibular lip but generally open to the tectorial membrane on the Reissner membrane side (Fig. 1a).

The tectorial membrane was thin on the vestibular lip and thick between the vestibular lip of the limbus and the organ of Corti (Fig. 1a).

**Scanning electron microscopy**

The mouse cochlear duct consisted of a hook and a spiral portion with one and half turns (Fig. 2). The surface of the connective tissue on the primary osseous spiral lamina was clearly exposed following removal of the cochlear duct epithelium (Figs. 2, 3). The surface was smooth due to the remaining basement membrane covering the connective tissue ele-

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**Fig. 2.** SEM views of the cochlea after exposing the connective tissue surface. VL and TL vestibular lip and tympanic lip of the spiral limbus, H hook, BM basilar membrane, SL spiral ligament. a. Oblique view of the cochlea. The central axis of the spiral is the modiolus. ×60. b. Top view of the modiolus. The vestibular lip (VL) shows a spiral row of auditory teeth. ×90
Fig. 3. Top views of the spiral limbus. The auditory teeth are demonstrated on the vestibular lip. The shape and arrangement of the teeth and the interdental furrows and spaces show regional differences. R base of the Reissner membrane. Habenular openings (H) are arranged along the edge of the tympanic lip of the spiral limbus. ×240. a. Apical end of the spiral limbus. b. Third half turn. c. Second half turn. d. First half turn. e. Hook. f. Basal end of the spiral limbus.
ments. The auditory teeth appeared on the upper convex surface of the spiral limbus between the edge of the vestibular lip and the base of the Reissner membrane (Figs. 2, 3). The surface of the inner spiral sulcus between the vestibular lip and tympanic lip was concave (Figs. 5, 6c). The surface from the sulcus to the tympanic lip was flat (Figs. 2, 3) and showed habenular openings aligning the edge of the spiral lamina at the junction with the basilar membrane (Fig. 3).

In the tooth zone, the teeth were different in shape between the outer and inner margins (Figs. 3, 4a). The teeth forming the vestibular lip possessed elongated upper plates separated by parallel slits and furrows for the interdental cells; a continuous row of these teeth resembled the keyboard of a piano (Figs. 3, 4, 5a, 6c). The upper plates of these teeth were about 8 μm in width, varying from 10 to 70 μm in length (Fig. 8). The teeth on the side of the Reissner membrane resembled mushrooms with star-shaped upper plates (Figs. 3, 4a, 6c), 5-25 μm in diameter (Fig. 8); they were separated by slits and gaps for the interdental cells. They were usually larger and longer toward the vestibular lip and smaller toward the base of the Reissner membrane. The upper plates of the teeth showed shallow linear grooves on the surface (Fig. 4b). The grooves on adjacent teeth delineated polygons, thus forming a network pattern across the slits which ran through the center of the polygons (Fig. 4b).

The edge of the vestibular lip was about 30 μm above the surface of the tympanic lip. Just below the edge, the concave surface of the inner spiral sulcus showed ovoid openings between the teeth (Fig. 5), measuring about 5 μm wide and 20 μm high, leading to tunnels for the arrays of the interdental cells (Fig. 6a, d). The tunnels ran parallel under the slits between the elongated upper plates of the teeth on the vestibular lip side and continued to the gaps between the star-shaped upper plates on the Reissner membrane side (Fig. 6a, b). The inner surface of the tunnels and gaps were smooth because of the covering basement membrane.

Regional differences in the tooth zone and spiral limbus

The elements of the spiral limbus varied continuously from the beginning of the hook to the apical end of the hamulus of the spiral lamina (Figs. 3, 5).

The width of the tooth zone was about 135 μm at
Fig. 5. Obliquely frontal views of the spiral limbus. VL edge of the vestibular lip, arrows openings of the tunnels for the interdental cells, TL tympanic lip, H habenular openings. a. Hook. The upper surface of the vestibular lip is convex. The internal sulcus is deeply concave. ×400. b. Apical end. The internal sulcus is shallow. ×550

Fig. 6. Spiral limbus after styrene resin cracking. AT bases of the auditory teeth. ×800. a. Cut face exposing the parallel, radially oriented tunnels (asterisks) between the teeth under the upper plates on the vestibular lip side. The inner sulcus is seen along the lower margin of this picture. b. Cut face exposing the randomly oriented spaces (asterisks) between the teeth under the upper plates on the Reissner membrane (R) side. The spaces are continuous with the tunnels on the vestibular lip side. c. Cross-cut view of the limbus. VL edge of the vestibular lip. d. Cut face across the teeth in the vestibular lip. The cross sections of the tunnels (asterisks) between the teeth are demonstrated.
its beginning, markedly decreasing in the hook to 100 μm, then gradually decreasing in the first and second half turns, and again markedly decreasing in the third half turn to measure about 20 μm at the apical end of the hamulus (Fig. 7). In the tooth zone, the length of the upper plates of the teeth on the side of the vestibular lip was 25–70 μm in the hook and first half turn, then decreasing toward the apex to measure 10–45 μm in the apical half turn (Fig. 8). The teeth with star-shaped upper plates on the side of the Reissner membrane were densely accumulated in the hook and first half turn, decreasing in density toward the apex; in the third half turn the teeth were sparsely distributed, thus widening the gaps for the interdental cells between the teeth (Figs. 3, 8).

The proportion of the area occupied by the interdental spaces in the hook and first half turn was small at each address across the tooth zone from the edge of the vestibular lip to the Reissner membrane, and markedly increased just before the base of the Reissner membrane (Fig. 9). In the second and third half turns, the proportion was small across the outer half of the tooth zone but markedly increased across the inner half to the base of the Reissner membrane (Fig. 9). The proportion of the area occupied by the teeth in the tooth zone was about 75% in the hook and first half turn and 55% in the second and third half turns.

The width of the tympanic lip between the edge of the vestibular lip and the edge of the spiral lamina in the top view of the cochlea was about 15 μm in the hook, increasing gradually in the first and second half turns and markedly in the third half turn, and measured 90 μm at the apical end (Fig. 7). The habenular openings along the edge of the spiral lamina were periodically arranged at a distance of 8 μm from the basal end of the hook to the apical end of the hamulus.

The width of the tympanic lip between the edge of the spiral lamina and the base of the Reissner membrane (the sum of the upper two widths) was about 150 μm at the basal end, decreasing in the hook to about 120 μm, and then constant to the hamulus where it decreased to 100 μm (Fig. 7).

The upper convex surface of the spiral limbus and the concave surface of the inner spiral sulcus in most parts of the spiral were respectively flattened or shallowed to the hamulus (Fig. 5).

DISCUSSION

Transmission electron microscopy has demonstrated that the interdental cells between the auditory teeth in the spiral limbus of the cochlear duct are bottle-shaped; they have a rounded body lying in the interdental furrow, a narrow neck between the upper plates of the teeth, and an apical cytoplasmic sheet lying on the upper surface of the tooth plate under the tectorial membrane; most of each cell is surrounded by the basal lamina and the stroma of the auditory teeth (IURATO, 1962; ILBERG, 1968; ISHIYAMA et al., 1970; LIM, 1970; THORN et al., 1979). It is believed that the smooth surface of the auditory teeth shown by SEM after removal of the interdental cells is the exposed basal lamina, and the contours of the auditory teeth indicate those of the interdental cells or cell cords as follows.

On the vestibular lip side of the spiral limbus, the interdental cells are arranged in simple rows between the elongated teeth. The slits between the upper plates of the teeth suggest that the necks of the cells are like thin plates, rather than poles as generally thought, and continue to the apical sheets covering the upper plates of the teeth. These plate-like necks may be so arranged as to form belts in the region of long tooth plates and a honeycomb-like network in the region of star-shaped tooth plates. The polygonal grooves on the upper surface of the teeth indicate the outline of apical sheet of each cell; the margins of the sheet may be firm and thick for the junction with neighboring cell sheets (FRANKE, 1978). On the Reissner membrane side, the shapes of the cells filling the
larger interdental spaces are like beakers with wide necks or cups with no neck.

It has been suggested that the interdental cells form and maintain the tectorial membrane by secretions consisting mainly of glycoproteins (Iurato, 1962; Voldrich, 1967; Ishiyama et al., 1970; Lim, 1970; Mira, 1971; Arnold and Vosteen, 1973; Thalmann et al., 1987). Ultrastructural studies suggest that the secretory materials arise in the supranuclear cytoplasm of the cells and are transported through the neck to the apical surface (Iurato, 1962; Ishiyama, 1970; Lim, 1970; Arnold and Vosteen, 1973). The differences in the shape of the neck portion of the interdental cells may be related to the transport of secretions; the wide neck region works more efficiently to transport secretions, compared to the narrow neck of the cells. Thus, the interdental cells on the Reissner membrane side are considered to be primarily involved in the secretion of the tectorial membrane materials.

Fig. 8. The relationship between the length and the location of the teeth in the tooth zone. The values are obtained from 140 µm long segments of the tooth zone in the hook (H), and the first (1), second (2) and third (3) half turns of the cochlear spiral. The horizontal axis shows the width of the tooth zone; the location of each tooth is indicated by the distance between the edge of the vestibular lip and the outermost point (dots) of the tooth. The longer teeth are accumulated in the outer half of the zone and the shorter ones in the inner half. The length of the teeth in the outer half, the number of the teeth in the inner half, and the width of the tooth zone all decrease from H to 3.

Fig. 9. The proportionate area between the upper surface of the teeth and the interdental spaces at given addresses across the tooth zone. The addresses are marked on the top view images of 140 µm long segments of the tooth zone in the hook (H), and the first (1), second (2) and third (3) half turns. The horizontal axis shows the address in the tooth zone as the distance from edge of the vestibular lip; the y-axis coincides with the edge of the vestibular lip and the vertical bars represent the base of the Reissner membrane. The proportionate area of the interdental spaces in the outer half of the tooth zone is small in the hook and every half turn but the area of the spaces in the inner half increases from the hook to the third half turn. The spaces between the teeth widen rapidly near the base of the Reissner membrane in the hook and the first half turn, while in the second and third half turn the spaces widen from the center of the zone and occupy the greatest area near the Reissner membrane.
The size of the tectorial membrane also increases from the base to the apex of the cochlear duct (Steel, 1983; Lim, 1986). In the present study, the gradation in the population density of the auditory teeth indicates a base-to-apex increase in the population of the interdental cells with wide necks on the Reissner membrane side. It is suggested that the interdental cells increase the secretion toward the apex to produce the tectorial membrane, which is large on the apical side of the cochlear duct.

The tectorial membrane is supported by the scaffold of the auditory teeth in the spiral limbus; the membrane is attached to the upper plates of the teeth, projects from the vestibular lip and remains free at the outer margin to cover the organ of Corti making contact with the stereocilia of the inner and outer hair cells (Hoshino, 1974; Lim, 1986). When the basilar membrane vibrates with sound waves, the tectorial membrane swings like a book-cover hinged at the edge of the vestibular lip, stimulating the hair cells (Davis, 1958; Bekesy, 1960). The large upper plates of the teeth in the vestibular lip may provide firm support for the tectorial membrane during its vibrations. The presence of fibers in the spiral limbus for anchoring to the osseous spiral lamina also indicates such a function (Iurato, 1962; Kucuk and Abe, 1989).

We demonstrated that the teeth on the vestibular lip side orient their long axis radially in the direction from the modiolus to the organ of Corti. By manipulating the tectorial membrane with microprobes, Frommer (1982), Zwislocki (1986) and Zwislocki et al. (1988) showed that the membrane on the spiral limbus is highly elastic; on the organ of Corti the membrane is firmly attached to the tip of such rigid stereocilia as can hardly be deflected on the hair cells (Lim, 1986; Strelloff and Flock, 1984). The membrane may be stretched in the radial direction from the limbus to the hair cells when the basilar membrane and hair cells are displaced toward the scala tympani during vibrations (Frommer, 1982; Zwislocki, 1986, Zwislocki et al., 1988). Such stretching of the tectorial membrane probably creates radial forces on the teeth. The orientation of the teeth, thus, seems to be adequate for maintaining the stabilization of the teeth during the radial stretching of this membrane.

We have shown that the teeth supporting the tectorial membrane are well developed on the basal side and less developed on the apical side of cochlear duct. Tiedemann (1971) and Strelloff et al. (1985) have indicated that the tectorial membrane and basilar membrane vibrate with high frequency sound waves at the base and with low frequency sounds at the apex of the cochlear duct. It is understandable that the tectorial membrane vibrating in high frequency at the base should be fixed to a well-developed support that can endure a greater mechanical stress created by the rapid vibrations. The length gradation of the teeth in the vestibular lip is considered to be closely related to regional differences in the strength of the support and vibratory pattern of the tectorial membrane.

The width of the tympanic lip is considered to be parallel to the width of the tectorial membrane. Thus the present study suggests a linear increase in the width of the tectorial membrane from the base to the apex. The stiffness of the basilar membrane also shows linear gradation along the cochlear duct owing to the amount of fibers in this membrane; the basilar membrane is thick and stiff in the base and thinner and less stiff in the apex (Bekesy, 1960; Tiedemann, 1971; Lim, 1986). Such gradations of the basilar membrane are generally believed to determine the regional differences in its vibration pattern (Bekesy, 1960). Thus, the gradations of the tectorial membrane coupled with the basilar membrane is considered to have significance on the regional hearing mechanics of the cochlear duct, as recently postulated by modeling studies of Strelloff et al. (1985) and Zwislocki (1986).

In conclusion, SEM of the connective tissue elements of the spiral limbus is useful for the understanding of the morphology and function of the tectorial membrane and the interdental cells. The structural variations of the spiral limbus suggest the presence of regional differences in the production and mechanics of the tectorial membrane in the cochlear duct.

REFERENCES


