ORIGINAL ARTICLE

Scanning Electron Microscopy of Auditory Ossicles

Ivan Zenev, MD; Emil Zenev, MD; Nikolaf Sapundzhiev, MD; Mario Milkov, MD; and Dimitrav Marev, MD

From the Department of Otolaryngology, Head and Neck Surgery, Queen Yoanna University Hospital of Sofia, Sofia, Bulgaria (I. Zenev); the Department of Otolaryngology, Head and Neck Surgery, Zentralklinikum Augsburg, Germany (E. Zenev); and the Clinic of Otorhinolaryngology, St Marina University Hospital of Varna, Varna, Bulgaria (N. Sapundzhiev, M. Milkov, and D. Marev)

Correspondence
Mario Milkov, MD
BG-9002 Varna St

Phone: +359-52-645025
Fax: +359-52-650019
E-mail: mariomilkof@yahoo.com

Submitted: 01 February, 2006
Accepted: 09 May, 2006

Mediterr J Otol 2006; 3: 112-116

OBJECTIVE: Middle ear auditory ossicles and the tympanic membrane are the morphologic substrate responsible for the transmission and conversion of environmental sounds to the internal ear.

MATERIALS AND METHODS: The ultrastructure of human complete ossicular chain specimens was morphologically studied with a scanning electron microscope.

RESULTS: Various essential morphologic details of the components of the ossicular chain in the middle ear were revealed. The auditory ossicles were covered with a fine complex mucoperiosteal layer, rich in capillary blood vessels, that formed complementary protrusions on the ossicular surface. Both the malleus and the incus consisted of separate osseous parts. The bone substance of the incus long process had a fibrous structure, and the incus short process was composed primarily of lamellar bone. The length of the malleus manubrium was 1.3 times that of the long process. The stapes footplate demonstrated separate embryologic development, and its surface facing the oval window was covered by a thin cartilaginous layer.

CONCLUSION: All surgical interventions performed on the ossicles should accommodate anatomic peculiarities as well as the articulations between and the connections of the ossicles with the tympanic membrane. Extensive knowledge of those characteristics is essential to ensure a successful outcome of functional middle ear surgery.

The modern otologic surgical armamentarium includes various tympanoplastic techniques and approaches for performing ossicular chain reconstruction, all of which are subject to a great variety of modifications. To achieve good postoperative results in patients who require middle ear surgery, it is essential to know and consider the construction of the ossicular chain and the structure and position of its elements as well as the physiologic process of hearing. From an ontogenetic point of view, those elements are of mesenchymal origin, which explains some common peculiarities of their structure. The tympanic membrane and the ossicles are undoubtedly the most important structures in the portion of the middle ear that is subjected to surgery. The objective of our study was to explore and chronicle the ultrastructure of the auditory ossicles by means of scanning electron microscopy.

MATERIALS AND METHODS

We conducted a morphologic study of human complete ossicular chain specimens. Ossicle explantation was performed after the entire temporal bone had been removed. After prefixation in cialit solution for 48 hours, the entire ossicular complex was cut and dissected. The first gross cut opened the vestibulum of the inner ear from its medial aspect. The next approach was made through the tympanostomy. Care was taken to preserve the integrity of the tympanic membrane and the entire annulus. After exposing the malleus and incus, we cut the supporting ligaments to the middle ear chamber walls and then transected the tympanic and the stapedius muscles. Thus the entire complex, which consisted of the tympanic membrane (with the annulus and remnants of the channel skin) and the ossicular chain, was fixed only to the oval window and the footplate. The ligament was carefully dissected as slight pressure was applied to the footplate on its medial surface from within the inner ear toward the middle ear. This dissection method was chosen to preserve the integrity of the chain and the interossicular articulation.

The entire complex was then further fixed and prepared for evaluation via scanning electron microscopy. The ossicles were first preserved for 6 months in Cialit 1:500 solution. Ten samples were immersed in 2% cold glutaraldehyde and were fixed for 3 hours. They were then transferred to a buffer solution for washing and were postfixed by 2% osmium tetroxide in the same buffer. After dehydration in ethanol, some samples were dried to the critical point, coated with gold chloride (10-15 nm), and observed under a Hitachi S-405 (Sapporo, Japan) scanning electron microscope.

RESULTS

Our results showed that the auditory ossicles are covered with a fine complex mucoperiosteal layer that forms various adhesions along the walls of the tympanic cavity. This layer is rich in the capillary blood vessels of the ossicles and the tympanic membrane (Figure 1). The stapes has a similar structure[1]. The mucosa of the long incudal process is smoother than that of the short incudal process. It is noteworthy that the footplate has a separate embryologic development. The surface of the stapes plate facing the oval window is covered by a thin cartilaginous layer. This structure is believed to demonstrate osteosclerotic processes at later stages in

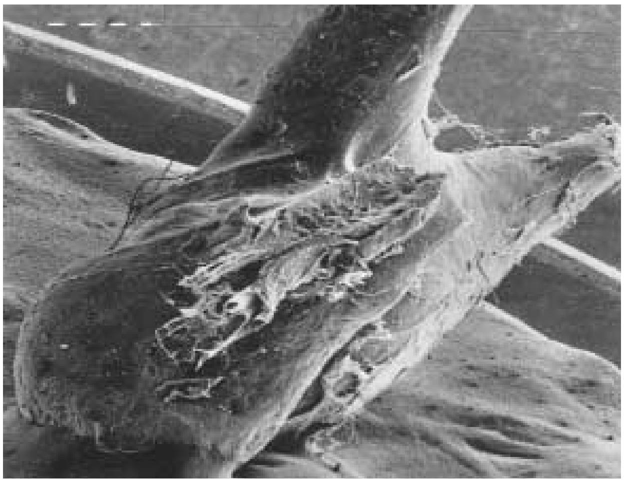

Figure 1: The corpus incudis. Scanning electron microscopy, original magnification _60. The short process, its mucosal cover, and the long process are well outlined. The visible surface is located in the fossa incudis, the mucosa of which is lacerated. In the left lower area, the rim of the incudomalleolar joint can be seen. The mucosa of the long incudal process located at the top right is smoother than that of the short incudal process.

the patient's life[2]. The thin mucosa around the incudomalleolar joint is lacerated (Figure 2). Preserving the incudomalleolar and incudostapedial articulations during tympanoplasty and stapedioplasty is crucial.

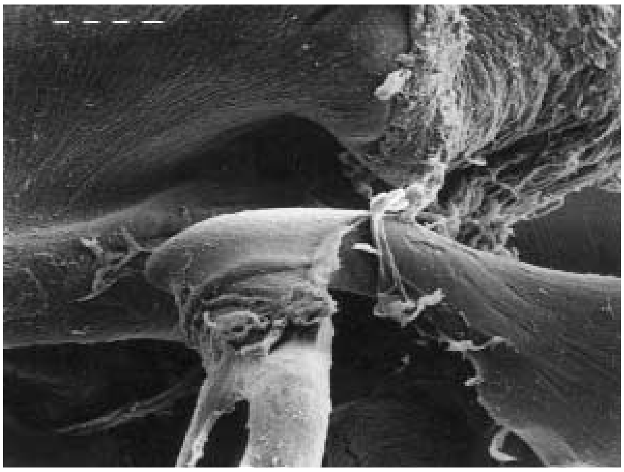

Figure 2: The articulatio incudomalleolaris. Scanning electron microscopy, original magnification _60. The joint, processus lenticularis, and thin mucosa (which is lacerated and bent backward) are visible.

The contact between the manubrium mallei and the tympanic membrane is quite solid (Figure 3), and the surface of the incudostapedial joint cartilage is relatively smooth. The junction is especially firm around the umbo, where the radial fibers fan out to attach along the inner margin of the tympanic bone (the annulus tympanicus). The mucosa of the remainder of the tympanic membrane is smooth (Figure 4). Important mechanical effects are produced by sound waves in the area of the umbo[3]. The resonance frequency of the umbilical part of the tympanic membrane is about 2400 Hz. The largest degree of movement is observed there, because that area serves as the fulcrum of the lever system of both the malleus and the incus, which act as second-order levers. The length of the manubrium mallei is 1.3 times as long as that of the incus; thus, the force is increased by 1.3 times. The ratio of the area of the tympanic membrane to that of the stapedial footplate is 18.6:1. Thus the last bone (the stirrup) moves with about 20 times the force of the hammer as a result of the lever principal.

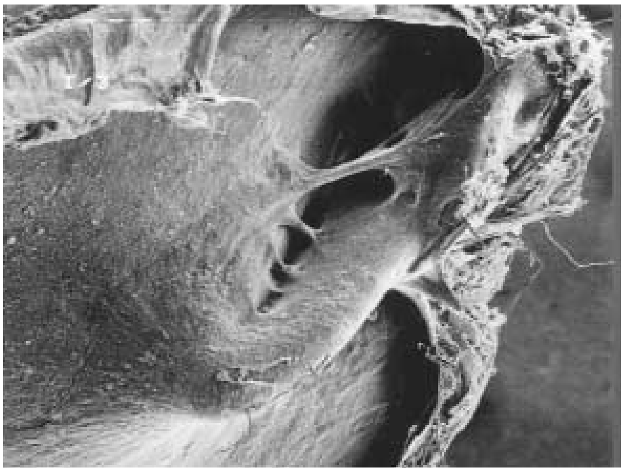

Figure 3: The surface of the incudostapedial joint. Scanning electron microscopy, original magnification _100. A portion of the ligament formed by fine connective tissue fibers can be seen. The joint cartilage surface is relatively smooth.

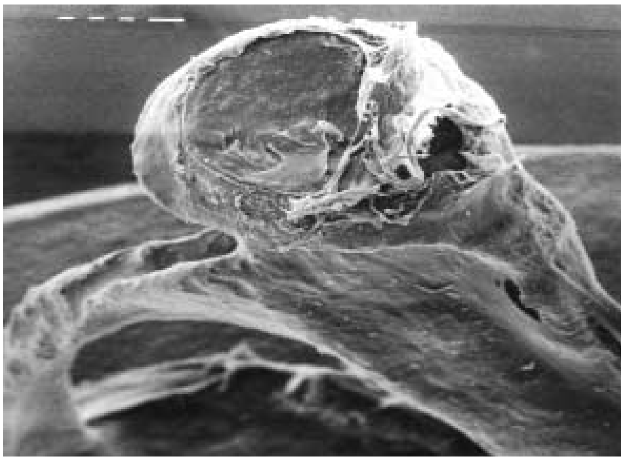

Figure 4: The manubrium mallei. Scanning electron microscopy, original magnification _60. This view shows the area around the umbo from the tympanic side of the tympanic cavity. The mucosa of the remainder of the tympanic membrane is smooth.

The integrity of the incus is an important anatomic prerequisite for the surgical restoration of the normal function of the middle ear. The length of that ossicular body plus that of the long process totals about 6.8 mm. The distal part of the lenticular process (the os sylvii) ends in a small knob positioned at an angle of 100 degrees relative to the main axis. The long incus limb and the lenticular apophysis are also covered with a complex mucoperiosteal layer that projects into the adjacent structures of the ossicles and the middle ear cavity. These mucosal folds are remnants of resorbed embryonal tissues that once filled the primitive fetal middle ear cavity. They include the nourishing vessels of the ossicle and are thus termed the "mesenterium cavi tympani". The interruption of the mesenterium cavi tympani during middle ear surgery could compromise the normal blood supply to the ossicle, its resistance and/or integrity, and the postoperative results.

DISCUSSION

Our results show that on scanning electron microscopy, the ossicles, the incudostapedial joint, and the site of contact between the manubrium and the tympanic membrane represent a complex system of limited mobility in the articular apparatus. Damage to any of the incudomalleolar and incudostapedial articulations subsequently results in ankylosis[4], which in turn causes a reduction in the acoustic gain by about 40 dB and a severe impairment of both normal sound conduction and transformation along the ossicular chain.

The inferior surface of the lenticular apophysis has an articulation surface for the incudostapedial joint with the head of the stapes. Even with its tiny dimension, this joint has morphologic characteristics similar to those of any joint and is surrounded by a fine fibrous capsule. Because this capsule is extremely fragile and vulnerable, it could easily be damaged during middle ear surgery or cochlear implantation, and such trauma could lead to partial or complete luxation[5]. A case of cholesteatoma and isolated erosion of the short process of the incus with a slight conductive hearing loss that remained unchanged 2.5 years after surgery has been reported[6]. The short process may contribute to hearing up to 10-15 dB and plays a role in epitympanic aeration by supplying an attachment surface to the incudal folds.

The limb of the long incus has another important role in reconstructive middle ear surgery. This is the site at which middle ear prostheses or implantable hearing aids (MedEl Vibrant Soundbridge) are fixed. A very firm fixation could cause ischemic necrosis of the distal part of the long process and thus impaired or discontinued sound conduction. However, if the wire loop of the prosthesis around the limb is too loose, it will induce friction at the fixation point and will thus cut through the long limb. Impaired or discontinued sound conduction will result. These 2 pathologic mechanisms could occur in patients with an implanted hearing aid. Even though the fixing clips of the Vibrant Soundbridge are placed on a much larger area of the long incus process, that hearing aid exerts a greater mechanical effect; its vibrator can sustain frequencies as high as 130 dB, which in turn may interfere with normal ossicular nourishment[7].

With these factors in mind, we created a modified middle ear prosthesis designed to ensure optimal sound conduction and maximum preservation of bone nourishment at the place of fixation on the incus. The prosthesis was made of fluoroplastic material. On the inner contour of the fixation loop, there were 4 wells designed to provide stable fixation while protecting areas of the mucoperiosteal layer from contact or pressure, thus preserving a level of blood circulation within normal limits. The initial performance of this new modified prosthesis was encouraging[8].

Recently, several publications have emphasized the role of scanning electron microscopy in middle ear surgery and in the pathologic analysis of middle ear disorders. One report described the microscopic, histologic, and scanning electron microscopic analysis of a stapes obtained when the bony fixation of the titanium prosthesis caused its accidental extraction during revision tympanoplasty[9]. The bony fixation of the lower surface of the prosthesis foot to the stapes footplate was confirmed by ultrastructural analysis[9]. During a scanning electron microscopic examination in another study, the integrity of the stereocilia of the inner and outer hair cells demonstrated no histologic evidence of significant cochlear damage, but a mild local middle ear inflammation caused by treatment with ciprofloxacin was noted[10]. Light microscopy and scanning electron microscopy have been used to determine morphologic changes in situ after ossiculoplasty in which total ossicular replacement prostheses were implanted into the tympanic cavities of rabbits[11]. The surfaces of aural polyps[12] and the tissue surrounding prematurely extruded ventilation tubes[13] have also been studied with scanning electron microscopy. The surface structures of gold, Teflon, and titanium, all of which are used in stapes prostheses, have been compared via scanning electron microscopy, and the results of that comparison suggest that the titanium prosthesis is the most suitable for use in stapes surgery[14]. Schwager examined 12 partial ossicular replacement titanium prostheses and 11 total prostheses via scanning electron microscopy and established that titanium is a biomaterial suitable for use in ossicular chain reconstruction[15]. The morphologic and microchemical changes affecting sclerotic stapes in patients with otospongiosis or van der Hoeve's syndrome have also been evaluated with a scanning electron microscope equipped with an energy-dispersive x-ray analyzer[16].

CONCLUSION

All surgical interventions performed on the middle ear auditory ossicles should accomodate anatomic peculiarities as well as the articulations between and the connections of the ossicles with the tympanic membrane. Our findings indicate that scanning electron microscopy reveals important morphologic details of the ossicular chain components and that extensive knowledge of those characteristics is essential to ensure a successful outcome of functional middle ear surgery.

REFERENCES

1. Zenev I, Betow C. Scanning electronic microscopy of sialit-preserved total middle-ear grafts. J Laryngol Otol 1985;99:1201-8.
2. Vick U, Just T, Terpe H, Graumuller S, Pau HW. [Stapes fixation in children] HNO 2004;52:1076-82. German.
3. Zenev I. Clinical and morphological otology. Sofia, Bulgaria: Marin Drinov Publishing House; 1999. Bulgarian.
4. Zenev I. Scanning electron microscopy of the auditory ossicles after preservation with sialit. Otorinolaringologiya (Sofia) 1982;19:22-8. Bulgarian.
5. Tucker AS, Watson RP, Lettice LA, Yamada G, Hill RE. Bapx1 regulates patterning in the middle ear: altered regulatory role in the transition from the proximal jaw during vertebrate evolution. Development 2004;131:1235-45. Epub 2004 Feb 18.
6. Aslan A, Aslan GG. Considerations of isolated destruction of the short process of the incus in a case of cholesteatoma. Eur Arch Otorhinolaryngol 2005;262:844-6. Epub 2005 Mar 1.
7. Langevin S. Surdite neurosensorielle: les implants d'oreille moyenne. Cah Bioméd 2004;165:25-30.
8. Zenev I, Kunev K, Dimov P, Rouev P. Stapeschirurgie mit bulgarischer Fluoroplasticprothese. HNO Inform 2005;30:84.
9. Sudhoff H, Lindner N, Gronemeyer J, Dazert S, Hildmann H. Study of osteointegration of a titanium prosthesis to the stapes: observations on an accidentally extracted stapes. Otol Neurotol 2005;26:583-6.
10. Kaplan DM, James AL, Thorp MA, Mount RJ, Harrison RV. Effects of middle ear application of Cipro HC Otic Suspension in an animal model. J Otolaryngol 2004;33:160-4.
11. Trabandt N, Brandes G, Wintermantel E, Lenarz T, Stieve M. Limitations of titanium dioxide and aluminum oxide as ossicular replacement materials: an evaluation of the effects of porosity on ceramic prostheses. Otol Neurotol 2004;25:682-93.
12. Skladzien J, Litwin JA, Nowogrodzka-Zagorska M, Miodonski AJ. Surface structures of aural polyps: a scanning electron microscopic study. J Laryngol Otol 2002;116:420-5.
13. Sammons RL, Burnett S, Pahor AL, Wilson C, Matthews JB. Histochemical, immunohistological and scanning electron microscope analysis of tissue retained on spontaneously extruded ventilation tubes. J Laryngol Otol 2002;116:333-9.
14. Kwok P, Fisch U, Strutz J, Jacob P. Comparative electron microscopic study of the surface structure of gold, Teflon, and titanium stapes prostheses. Otol Neurotol 2001;22:608-13.
15. Schwager K. [Scanning electron microscopy findings in titanium middle ear prostheses] Laryngorhinootologie 2000;79:762-6. German.
16. Vallejo-Valdezate LA, Martin-Gil J, Jose-Yacaman M, Martin-Gil FJ, Gil-Carcedo LM. Scanning electron microscopy images and energy-dispersive X-ray microanalysis of the stapes in otosclerosis and Van der Hoeve syndrome. Laryngoscope 2000;110:1505-10.