Middle ear structure and transcanal approach appropriate for middle ear surgery in rabbits

MING GUAN[1,2*], JIE ZHANG[3*], YUEZHI JIA[1,2], XIAOLIN CAO[1,2], XIANGYU LOU[1,2], YONG LI[1,2] and XIA GAO[4]

[1]Department of Otolaryngology, The Affiliated Hangzhou Hospital of Nanjing Medical University; [2]Department of Otolaryngology, Hangzhou First People's Hospital, Hangzhou, Zhejiang 310006; [3]Department of Pediatrics, Hangzhou Children's Hospital, Hangzhou, Zhejiang 310000; [4]Department of Otolaryngology, Nanjing Drum Tower Hospital, Clinical College of Nanjing Medical University, Nanjing, Jiangsu 210008, P.R. China

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Abstract. The current study aimed to investigate the middle ear structure and surgical approach appropriate for middle ear surgery in rabbits. A total of eight healthy New Zealand rabbits (16 ears) were dissected under a surgical microscope. The dimensions of the auditory canal and the middle ear were measured. In the present study, the transcanal surgical approach to the middle ear in rabbits was performed without complications, the anatomical landmarks in the auricle and the external auditory canal were apparent, no large vessels were present in the surgical zone and the bleeding was minor. Furthermore, the surgical procedure did not require removal of large bone sections of the external auditory canal. Additionally, the constitution of the ossicular chain, the leverage ratio of the ossicular chain and the constitution of ligaments and muscles in rabbits were similar to humans. Otherwise, the facial nerve canal in rabbits was more prominent compared with humans and the mobility of pars flaccida in rabbits was more noticeable compared with humans. The results of the current study indicate that the transcanal surgical approach was suitable to study the middle ear in rabbits. Furthermore, the rabbit middle ear may be used as a model for ossicular surgery and facial nerve research.

Correspondence to: Professor Xia Gao, Department of Otolaryngology, Nanjing Drum Tower Hospital, Clinical College of Nanjing Medical University, 321 Zhongshan Street, Nanjing, Jiangsu 210008, P.R. China
E-mail: chalise@126.com

Dr Yong Li, Department of Otolaryngology, The Affiliated Hangzhou Hospital of Nanjing Medical University, 261 Huansha Street, Hangzhou, Zhejiang 310006, P.R. China
E-mail: leeyung828@hotmail.com

*Contributed equally

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Introduction

Experimental models serve important roles in otological research, and animal models of the middle ear are required for otorhinolaryngologists to gain experience and competence, and to develop novel surgical techniques (1-3). The most valuable animal models exhibit the greatest degree of similarity with humans (4,5). At present, guinea pigs and rats are mainly used in otological research, including ototoxictiy research (6), sensorineural hearing loss research (7), cochlear implantation research (8) and gene therapy research (9). However, the tympanic cavity of these animals is frequently too small to perform middle ear surgeries, thus it has been hypothesized that larger animal models, including rabbits, sheep and pigs will more accurately reflect the anatomy of the human ear and it will be easier to perform middle ear surgeries (10-15). The rabbit middle ear has been widely used for middle ear surgeries in certain procedures, including osscicular chain restoration (11,16,17) and stapes surgery (18,19). The retroauricular surgical approach is the most frequently applied method in rabbit middle ear surgery (11). However, the anatomical structure of the rabbit middle ear has not been well documented and it has been reported that gaining access to this area via the retroauricular surgical approach is challenging (11,16). Thus, the current study aimed to investigate the middle ear structure in rabbits and to develop a surgical approach appropriate for middle ear surgery in rabbits.

Materials and methods

Eight New Zealand rabbits (age, 6 months; four males and four females; weight, 2.0-2.5 kg), were obtained from the Zhejiang Academy of Medical Sciences (Hangzhou, China). Animals were maintained in a conventional animal environment (temperature, 26±2˚C; humidity, 55±10%) with a 12-h light/dark cycle. Animals had free access to water and were fed three times per day. Rabbits were deeply anesthetized by intravenous injection of sodium pentothal (~30 mg/kg) into the auricular vein of the left ear. In addition, disappearance

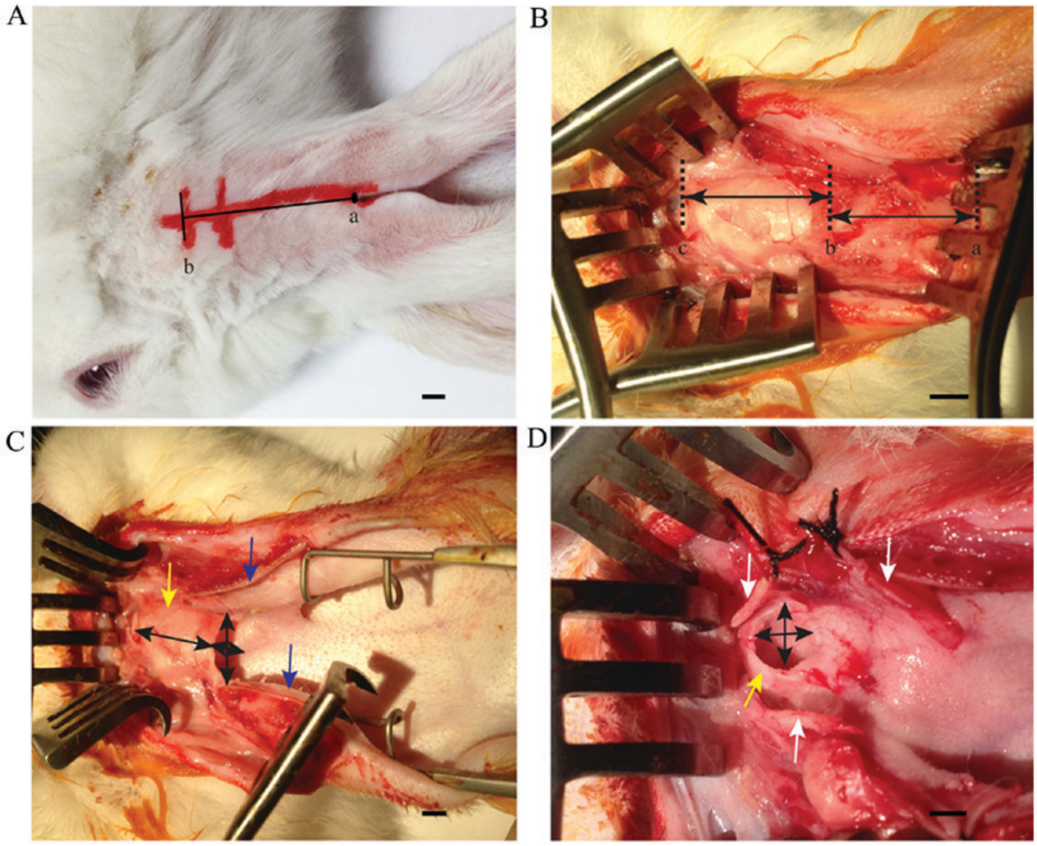

Figure 1. Anatomy of the external auditory canal and the surgical approach to the middle ear in rabbits. (A) An incision was made along the axis of the rabbit ear from the auricular notch (a point) to the connective line between the cartilaginous part and the bony part of the external auditory canal (b line). (B) The auricular cartilage (form a to b) and the cartilaginous part of the external auditory canal (from b to c) were exposed and measured. (C) The auricular cartilage (blue arrow) was opened. The aperture and the length (black arrows) of the cartilaginous part of external auditory canal (yellow arrow) were measured. (D) The bony part of external auditory canal (yellow arrow) was exposed following incision of the auditory canal cartilage (white arrows), and the aperture (black arrows) of the bony part of external auditory canal (yellow arrow) was measured. Scale bar=0.50 mm.

of the conjunctival reflex and the decrease in muscle tension of four limbs were used as signs to stop the injection. Rabbits were stabilized on a surgical table. The preauricular skin was shaved and disinfected with 5% iodine in 95% ethanol. First, an incision was made along the axis of the rabbit ear from the auricular notch to the connective line between the cartilaginous part and the bony part of the external auditory canal, and then the auricle and the cartilaginous part of the external auditory canal were dissected to clearly expose the tympanic membrane. Subsequently, the external auditory canal skin flap was dissected and the tympanic cavity was observed under the microscope. Lastly the posterior and superior walls of the bony external auditory canal were removed to investigate the anatomical structures in the rabbit middle ear. The surgical procedures were approved by the Institutional Animal Care and Use Committee of the Zhejiang Academy of Medical Sciences. Operations were performed under a surgical microscope and digital photomicrographs were captured using the attached camera. Anatomical structures were measured using a (Shang Guang, Inc., Shanghai, China), microscope graticules (Shang Guang, Inc., Shanghai, China) and Image-Pro Plus software (version 6; Media Cybernetics, Inc., Rockville, MD, USA). Data were analyzed using SPSS software (version 13.0; SPSS, Inc., Chicago, IL, USA).

Results

Anatomical structure of the external auditory canal and the surgical approach into the middle ear in rabbits. The transcanal surgical approach was applied to study the middle ear anatomy in rabbits through an incision along the axis of the rabbit ear from the auricular notch to the connective line between cartilaginous part and bony part of the external auditory canal (Fig. 1A). The length of the auricular cartilage and the auditory canal cartilage was measured (Fig. 1B). Subsequently, the connection between the auricular cartilage and the auditory canal cartilage was separated, and the auricular cartilage was cut to measure the aperture of the cartilaginous auditory canal (Fig. 1C). The auditory canal cartilage was cut to measure the aperture of the bony auditory canal (Fig. 1D).

Observation of the tympanic membrane in rabbits under a surgical microscope. The tympanic incisure in the posterosuperior part of the bony auditory canal was covered by pars flaccida, which was thick and exhibited movement with respiration (Fig. 2A). Pars tensa was semicircular and fixed, forming a sharp angle with the external auditory canal. This portion of the tympanic membrane was thin and tightly connected with the malleus, and the handle of malleus was observed through

Table I. External auditory canal structure in rabbits.

Structure	Dimensions (mm)
Length of the auditory canal auricular cartilagious part	19.94±0.41 (19.25-20.85)
Length of the auditory canal cartilagious part	19.23±0.82 (17.90-20.87)
Length of the auditory canal bony part	10.50±0.50 (9.50-11.10)
Height of the auditory canal cartilagious part	6.39±0.61 (5.34-7.34)
Transverse diameter of the auditory canal cartilagious part	8.96±0.55 (8.11-9.89)
Height of the auditory canal bony part	5.04±0.34 (4.45-5.74)
Transverse diameter of the auditory canal bony part	5.61±0.20 (5.25-6.03)

Data are presented as the mean ± standard deviation (minimum-maximum). N=16.

it (Fig. 2A). The interface between pars tensa and pars flaccida formed the anterior malleolar fold and the posterior malleolar fold, which were visible beyond pars tensa (Fig. 2A). After lifting the skin of the external auditory canal, the handle of the malleus and the chorda tympani nerve were observed (Fig. 2B). Furthermore, a part of the lenticular process of the incus and the incudostapedial joint were observed following adjustment of the microscopic angle (Fig. 2C). A part of the posterosuperior wall of the auditory canal was removed to completely expose the ossicular chain composed of malleus, incus and stapes (Fig. 2D).

Morphology of the rabbit ossicular chain. The structure of malleus included the head, neck, handle and anterior process. The end of handle was bent outward where the pars tensa connected (Fig. 3A). The incus consisted of the body, long foot, short foot and lenticular process (Fig. 3B). The stapes consisted of the head, neck, anterior foot, posterior foot and footplate (Fig. 3C). The stapedial footplate was arched, and its superior border was thick on both sides and thin in the middle (Fig. 3C). The articular surface of the malleus-incus joint was curved and tightly connected (Figs. 2D and 3A and B). The incudostapedial joint was formed between the lenticular process of the incus and the head of stapes and was easily separated compared with humans (Figs. 2D and 3B).

Ossicular ligament and ossicular muscle in the middle ear. After separating the incudostapedial joint and moving the auditory ossicular chain, the ligaments and muscles connecting the auditory ossicles could be observed, including the anterior ligament of malleus, posterior ligament of incus, annular ligament of stapes, tensor tympani and stapedius. The anterior ligament of malleus connected the end of the anterior process of the malleus to the superior wall of the tympanic cavity. Furthermore, the posterior ligament of the incus connected the end of the short process of the incus to the posterior wall of the incudal fossa located in close proximity to the facial nerve (Fig. 4A and B). Tensor tympani arose from the medial wall of the tympanic cavity located superior to the tympanic ostium of the eustachian tube and ended at the distal handle of the malleus (Fig. 4B). Stapedius arose from the posterior wall of the tympanic cavity and ended in the posterior region of the stapes neck (Fig. 4C). The annular ligament of stapes was located around the footplate and connected to the vestibular window (Fig. 4D).

Table II. Middle ear structure in rabbits.

Structure	Dimensions
Malleus lever arm	2.57±0.05 (2.51-2.62)
Incus lever arm	1.55±0.05 (1.49-1.60)
Malleus length	3.22±0.07 (3.08-3.32)
Incus length	2.49±0.03 (2.40-2.54)
Footplate length	1.44±0.01 (1.43-1.47)
Footplate width	1.10±0.02 (1.08-1.14)
Footplate area	1.14±0.02 (1.10-1.16)[b]
Footplate perimeter	4.04±0.03 (3.96-4.09)
Footplate thickness	0.18±0.01 (0.16-0.20)
Stapes foot angle	76.28±2.54 (72.41-80.82)[c]
Stapes height	1.02±0.01 (1.01-1.04)
Stapes head area	0.21±0.01 (0.20-0.23)[b]
Stapes head length	0.66±0.01 (0.64-0.68)
Stapes head width	0.46±0.02 (0.42-0.48)
Facial nerve canal diameter	0.79±0.03 (0.76-0.85)
Facial nerve canal length[a]	2.74±0.07 (2.60-2.89)

All dimensions are in mm unless otherwise stated. Data are presented as the mean ± standard deviation (minimum-maximum). N=16. [a]Length of the facial nerve canal was measured from the origin of tensor tympani muscle to the midline of stapes head. [b]Units, mm^2. [c]Units, degrees.

Facial nerve, vestibular window and tympanic ostium of the eustachian tube in the rabbit tympanic cavity. Following dislocation of the incus and stapes, and removal of the posterosuperior meatal wall, the facial nerve, vestibular window and tympanic ostium of eustachian tube were observed. The tympanic segment of the facial nerve was located superior to the vestibular window (Fig. 5A). The mastoid segment of the facial nerve was located posterior to the vestibular window and stapes (Fig. 5B). The vestibular window in the medial wall of the tympanic cavity was oval-shaped (Fig. 5C). The tympanic ostium of the eustachian tube was inferior to the semicanal of the tensor tympani, which was open like a horn mouth (Fig. 5A).

Measurement of anatomical structures in the rabbit ear. The anatomical structures of the external auditory canal in rabbits

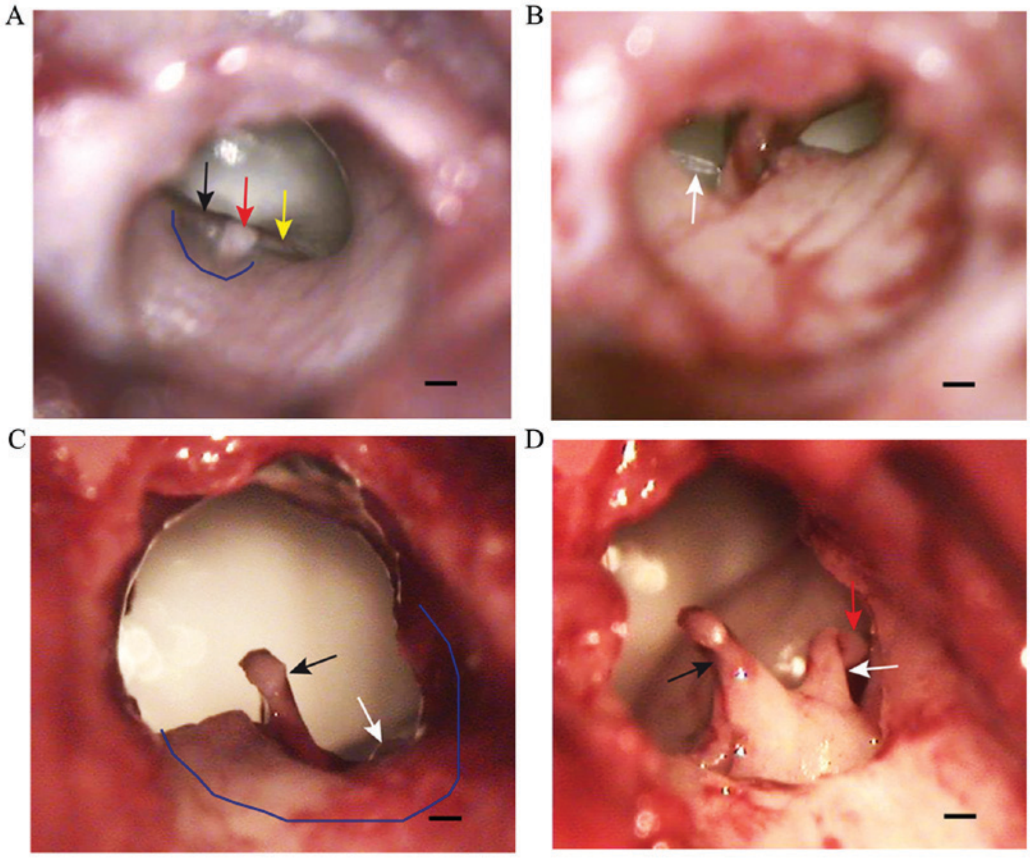

Figure 2. Anatomy of the middle ear in rabbits. (A) The anterior malleolar fold (yellow arrow), posterior malleolar fold (black arrow) and the handle of malleus (red arrow) were observed using a microscope through the external auditory canal. The tympanic incisure in the posterosuperior part of the external auditory canal was marked by a blue line and covered by the pars flaccida. (B) The chorda tympani nerve (white arrow) crossing under the malleus was observed after raising the skin flap of the external auditory canal. (C) The malleus (black arrow) and incus (white arrow). The posterosuperior bony part of the external auditory canal was marked by a blue line and was subsequently removed for complete exposure of the entire ossicular chain. (D) Following the removal of the marked bone, the ossicular chain, including the malleus (black arrow), incus (white arrow) and stapes (red arrow) was exposed. Scale bar=0.50 mm.

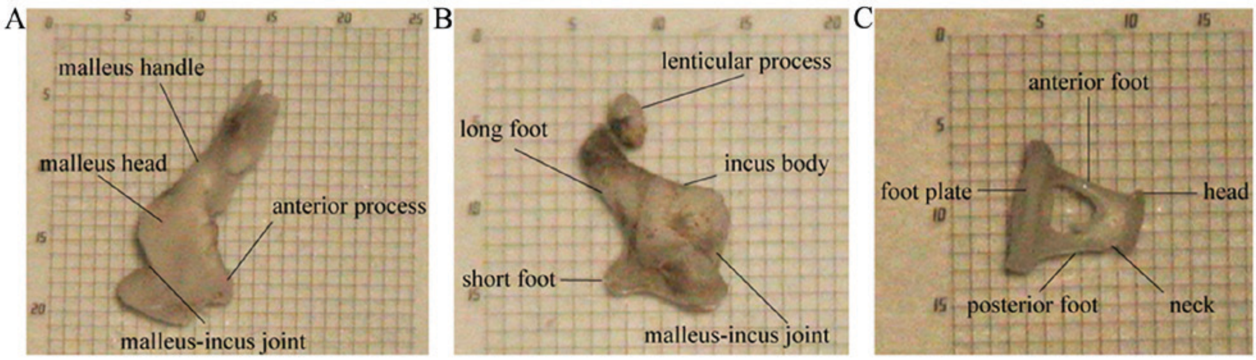

Figure 3. Morphology of the ossicular chain. (A) The structure of malleus included the head, handle and anterior process. (B) The structure of incus included the body, long foot, short foot and lenticular process. (C) The structure of the stapes included the head, neck, anterior foot, posterior foot and footplate. Although the stapedial footplate was arched and its superior border was thick on both sides and thin in the middle, the inferior border was uniform thick. Small square=0.2x0.2 mm.

were measured as indicated in Fig. 1, and data are presented in Table I. The results demonstrated that the length of the auditory canal auricular cartilagious part was 19.94±0.41 mm, the auditory canal cartilagious part was 19.23±0.82 mm and the auditory canal bony part was 10.50±0.50 mm. Additionally, the height and transverse diameter of the auditory canal cartilagious part were 6.39±0.61 and 8.96±0.55 mm, respectively, and the height and transverse diameter of the auditory canal bony part were 5.04±0.34 and 5.61±0.20 mm, respectively. In addition, the anatomical structure of the middle ear was measured as illustrated in Figs. 6 and 7, and the results are presented in Table II. The results demonstrated that the length of the lever arm of malleus was 2.57±0.05 mm, while the length of the lever arm of incus was 1.55±0.05 mm; therefore the leverage ratio of the ossicular chain was 1.66:1. The malleus length was 3.22±0.07 mm, the incus length

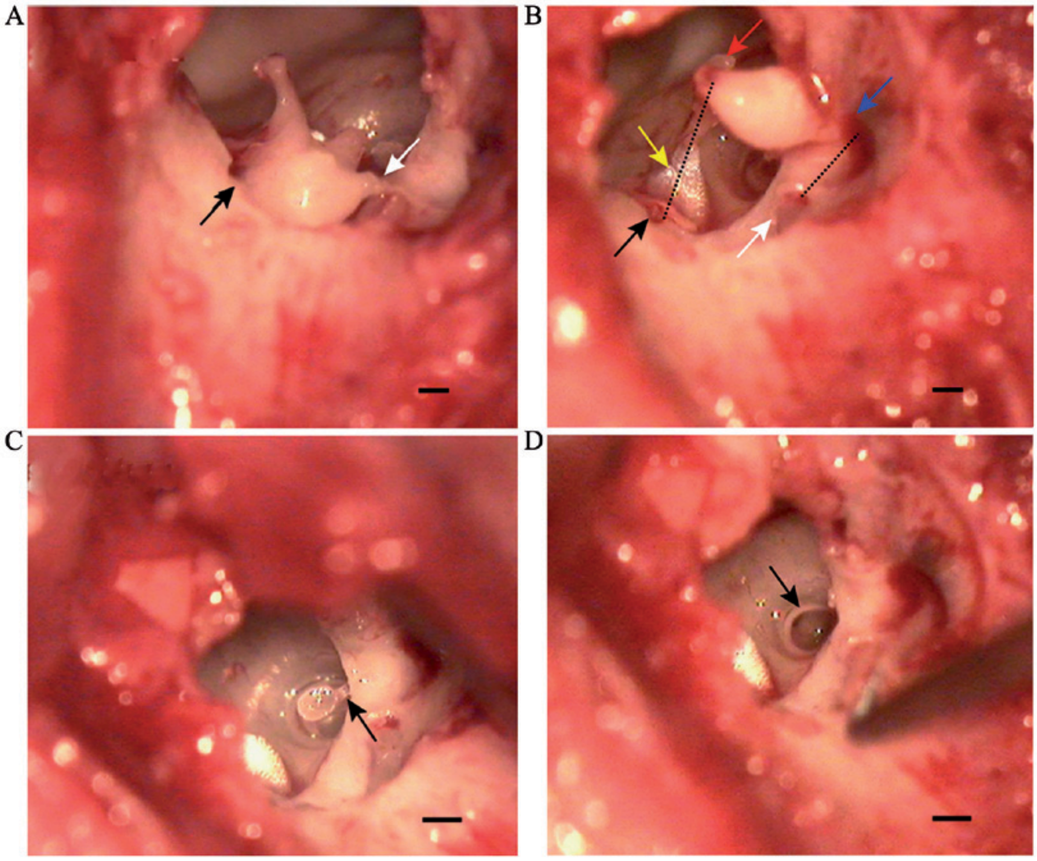

Figure 4. Ossicular ligaments and muscles in the rabbit middle ear. (A) The anterior ligament of the malleus (black arrow) and the posterior ligament of the incus (white arrow) were observed. (B) The anterior ligament of the malleus connected the anterior process of the malleus (red arrow) to the superior wall of the tympanic cavity (black arrow). The posterior ligament of the incus connected the short process of the incus (blue arrow) to the incudal fossa (white arrow) in close proximity to the facial nerve. Tensor tympani (yellow arrow) began at the medial wall of the tympanic cavity, and ended at the distal handle of the malleus. (C) The stapedius (black arrow) began at the posterior wall of the tympanic cavity, and ended in the posterior region of the neck of staples. (D) The annular ligament of the stapes (black arrow) was located around the vestibular window. Scale bar=0.50 mm.

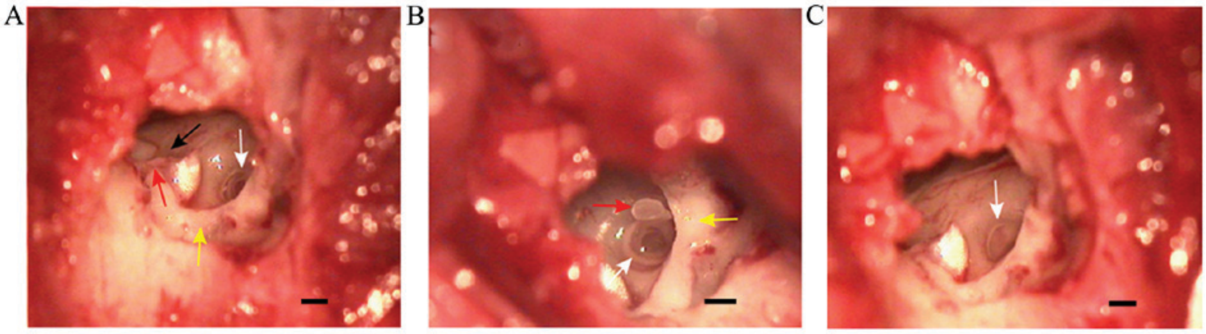

Figure 5. Facial nerve, vestibular window and the tympanic ostium of the eustachian tube in rabbit tympanic cavity. (A) The tympanic ostium of the eustachian tube (black arrow) located under the semicanal of the tensor tympani muscle (red arrow) was open like a horn mouth. The tympanic segment of the facial nerve (yellow arrow) was located superior to the vestibular window (white arrow). (B) The mastoid segment of the facial nerve (yellow arrow) was located posterior to the vestibular window (white arrow) and stapes (red arrow). (C) The vestibular window in the medial wall of the tympanic cavity was oval-shaped (white arrow). Scale bar=0.50 mm.

was 2.49±0.03 mm, and the footplate length, width, area, perimeter and thickness were 1.44±0.01, 1.10±0.02 mm, 1.14±0.02 mm^2, 4.04±0.03 and 0.18±0.01 mm, respectively. The stapes foot angle, height, head area, length and head width were 76.28±2.54˚, 1.02±0.01 mm, 0.21±0.01 mm^2, 0.66±0.01 and 0.46±0.02 mm, respectively. Additionally, the facial nerve canal diameter and length were 0.79±0.03 and 2.74±0.07 mm, respectively.

Discussion

The surgical approach to the middle ear was performed transcanally in the present study. Rabbit skin was cut vertically from the auricular notch to the boundary between the cartilaginous and the bony part of the external auditory canal. The auricle and the cartilaginous part of the external auditory canal were dissected to clearly expose the tympanic

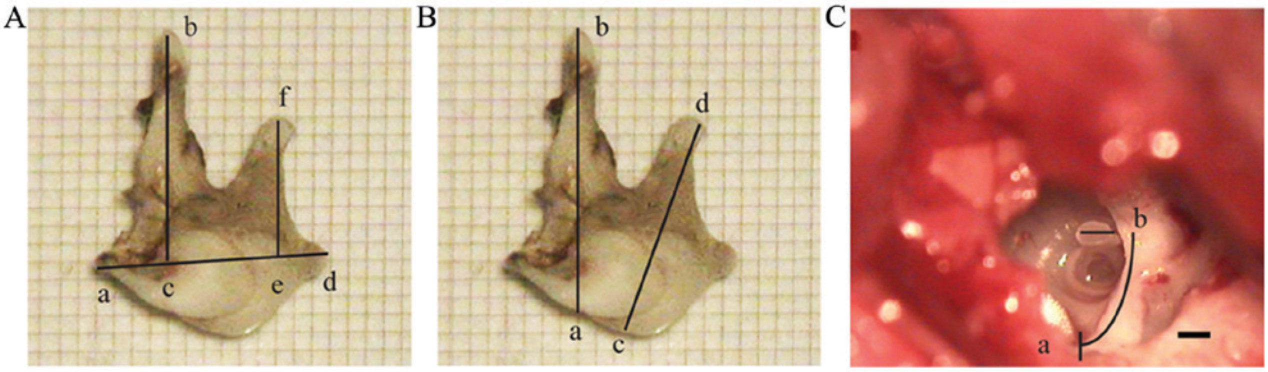

Figure 6. Vernier scale and Image-Pro Plus software were used to measure the anatomical structure of the middle ear in rabbits. (A) The distance between the anterior process of the malleus (a point) and the short process of the incus (b point). The malleus lever arm was measured as the perpendicular distance between the tip of the handle (d point) and the rotatory axis [the line between the anterior process of the malleus (a point) and the short process of the incus (b point)], and the incus lever arm was the perpendicular distance between the center of the lenticular apophysis (f point) and the rotatory axis. (B) The length of malleus was measured from the tip of the handle (b point) to the farthest point of malleus (a point) and the length of the incus was measured from the center of the lenticular process (d point) to the farthest point of incus (c point). (C) The length of the facial nerve canal between the origin of tensor tympani (a point) and the midline of the head of stapes (b point) was measured. Scale bar=0.50 mm. Small square=0.2x0.2 mm.

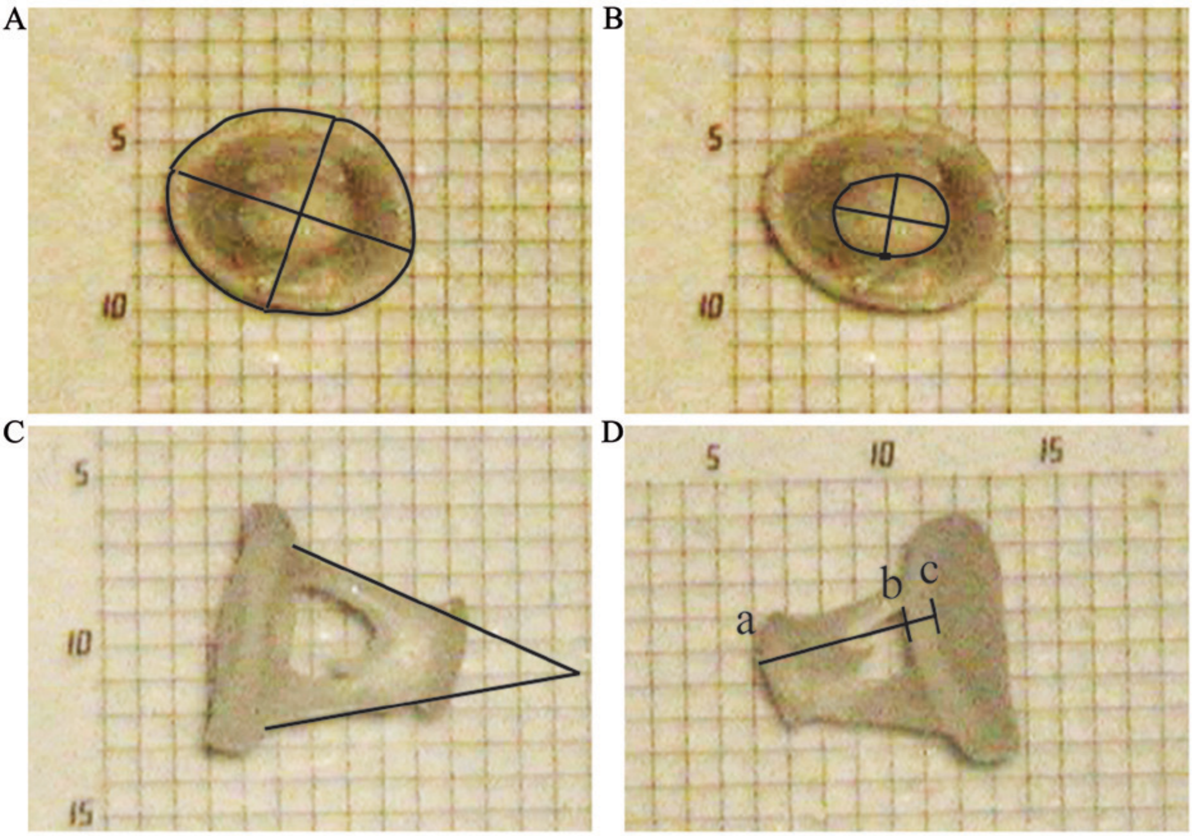

Figure 7. Vernier scale and Image-Pro Plus software were used to measure the structure of the stapes. (A) The measurement method of the footplate length, width, area and perimeter. (B) The measurement method of the length, width and area of the head of stapes. (C) The tangent line of the anterior and posterior foot was used to measure the angle between the anterior and posterior foot of stapes. (D) A vertical line from the middle point of the stapedial head (a point) intersect with the footplate at points b and c. The distance from point b to c was used to measure the thickness of the footplate and the height of the stapes was measured from point a to c. Small square=0.2x0.2 mm.

membrane. Secondly, we dissected the external auditory canal skin flap and observed the tympanic cavity under the microscope. The posterior and superior walls of the bony external auditory canal were removed to investigate the malleus, incus and stapes. These surgical procedures were performed without complications and have numerous advantages. The anatomical landmarks in the auricle and the external auditory canal were apparent, making it easily to locate the incision, which was along the axis of the rabbit ear from the auricular notch to the connective line between the cartilaginous part and the bony part of the external auditory canal, and repair the wound. No large vessels were present in the surgical zone and the bleeding during surgery was minor. Furthermore, removal of large bony sections of the external auditory canal or the mastoid was not required, which was performed in the retroauricular surgical approach but not the trancanal surgical approach, thus shortening the duration of surgery and avoiding severe tissue injury. There results indicate that the transcanal surgical approach was a suitable option to study the middle ear anatomy in rabbits.

The anatomical structure of the external and middle ear in rabbits was similar to the human anatomy (11); however, certain unique features were identified. The external ear could be separated into three parts, including the auricular cartilage part, auditory canal cartilaginous part and auditory canal bony part, respectively. The cross-section shape of the auditory canal was oval and markedly narrower inside than outside. The height of the cartilaginous part of the auditory canal was ~6.39±0.61 mm and the height of the bony part was ~5.04±0.34 mm (Table I). The tympanic membrane was hardly accessible unless the cartilaginous part of the auditory canal was opened and retracted.

The tympanic membrane in rabbits and humans consisted of two parts, including pars tensa and pars flaccida. Pars tensa in rabbits was fixed and formed a sharp angle with the external auditory canal, which was thin and tightly attached to the malleus, making it harder to separate them. Pars flaccida was moving with respiration, and, therefore, it was hypothesized that the eustachian tube may be constantly open in rabbits, and the movement of pars flaccida may balance the air pressure in the tympanic cavity. Furthermore, the authors of the current study hypothesized that the accessible eustachian tube increased the susceptibility of rabbits to media otitis, suggesting that rabbits may be used to establish animal models of media otitis. The interface between pars tensa and pars flaccida was more visible compared with the human equivalent, as the anterior malleolar fold and posterior malleolar fold projected beyond pars tensa. Furthermore, a tympanic incisure was identified in the posterosuperior zone of the bony part of the external auditory canal covered by pars flaccida.

The incus and stapes remained invisible until the posterosuperior bony wall of the auditory canal was partially removed. The connection between the incus and stapes was easily discontinued, enabling the dislocation of the stapes from the vestibular window. Therefore, the auditory ossicles should be manipulated with care.

The constitution and leveraging capabilities of the middle ear ossicles in rabbits were similar to humans. As in humans, the ossicular chain in rabbits was composed of the malleus, incus and stapes. The shapes of these three auditory ossicles in rabbits and human were also quite similar. The structure of the malleus could be divided into the head, neck, handle and anterior process. The incus was composed of the body, long foot, short foot and lenticular process. Furthermore, the stapes consisted of the head, neck, anterior foot, posterior foot and footplate. The leverage ratio of the ossicular chain in rabbits was ~1.66:1, similar to the 1.31:1 ratio of the human ossicular chain (20). The area of the footplate in rabbits was ~1.14 mm^2, markedly smaller compared with 2.97-3.03 mm^2 in humans (21-23); however, larger compared with 0.79 mm^2 in guinea pigs (21). The ossicular chain in guinea pigs is fused at the incus-malleus level (24). Furthermore, the carotid artery in humans is located in anterior and inferior aspect of basal turn of the cochlea, whereas the carotid artery in rats passes along the base of the cochlea;, thus, exposure of the oval window by removing the stapes foot in rats can cause hemorrhages and animal mortality (25-27). The conduction of sound is the main function of the ossicular chain (27). Due to the similarities in the constitution, leverage ratio and morphology of the ossicular chain between rabbits and humans, rabbits may serve as a model for stapes surgeries, including stapedectomies and stapedotomies, and ossiculoplasties. This model may be used to investigate novel surgical techniques or test new materials for reconstruction of the middle ear.

Ligaments were also observed in the middle ear cavity of rabbits used in the current study. The anterior ligament of malleus connected the end of the short process of malleus to tegmen tympani. The posterior ligament of incus connected the end of the short process of incus to the posterior wall of incudal fossa located in close proximity to the facial nerve. The annular ligament of stapes connected the foot plate of stapes to the vestibular window. Furthermore, two muscles of auditory ossicles were identified in the middle ear cavity of animals used in the current study. Tensor tympani began at the medial wall of the tympanic cavity superior to the tympanic ostium of eustachian tube and ended at the distal handle of malleus. The stapedius began at the posterior wall of the tympanic cavity and ended at the posterior region of the neck of stapes. Like in humans, these ligaments and muscles in rabbits may contribute to the stabilization of the ossicular chain and protection of the inner ear from loud sounds.

The facial nerve canal was exposed following removal of the bony part of the posterosuperior wall of the auditory canal, as there was space between the mastoid segment of the facial nerve and the posterior wall of the external auditory canal. The facial nerve canal was prominent and identified easily. Therefore, the rabbit middle ear may serve as a model for facial nerve research, including facial nerve decompression and facial nerve grafting. The vestibular window in rabbits was located inferior to the facial nerve tympanic segment and anterior to the facial nerve mastoid segment as in humans, enabling its complete exposure.

In conclusion, the transcanal surgical approach to the middle ear in rabbits was performed without complications and was suitable to study the middle ear. The anatomical structure of the middle ear in rabbits was similar to the human anatomy; however, the facial nerve canal was more prominent and easily identifiable. The results of the current study indicated that the rabbit middle ear may serve as a model for ossicular surgery and facial nerve research.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

XG and YL designed the experiments. MG, JZ, YJ, XC, and XL performed the experiments. MG, JZ, YJ, XC, XL analyzed the data. MG, JZ, XG, and YL wrote the manuscript.

Ethics approval and consent to participate

Surgical procedures were approved by the Institutional Animal Care and Use Committee of the Zhejiang Academy of Medical Sciences (Hangzhou, China).

Patient consent for publication

Not applicable.

Competing of interests

The authors declare that they have no competing interests.

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