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# Micro-Architecture Study of the Normal Infant Auditory Ossicles with Micro-Computed Tomography

## Estudio de Microarquitectura de los Osículos Auditivos Infantiles Normales con Tomografía Microcomputarizada

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**SUMMARY:** This study aimed to investigate the micro-anatomical morphology of ossicular chain in term fetus using micro-CT, in order to analyze the parameters of internal ossicular structure that may affect sound conduction.Four ossicular chains from two term fetuses were scanned by micro-CT. The related structural parameters of the trabeculae within the incus and malleus were calculated and compared. The fine anatomical structure of the auditory ossicles was analyzed.The microstructure of each auditory ossicles in term fetuses was clearly revealed by micro-CT. A marrow cavity was observed in the incus and malleus. In statistical analysis of the structural parameters of trabeculae in the incus and malleus, significant differences were found in BS/BV and Tb.Th ($P < 0.05$). Micro-CT enables the visualization of internal ossicular structure. The auditory ossicles in term fetus has good bone quality. The obtained bone structure data will help to clarify the physiological functions of normal fetal auditory ossicles.

**KEY WORDS: Auditory ossicles; Trabecula; Micro-CT.**

## INTRODUCTION

The auditory ossicles are located in the tympanic cavity of the petrous bone. It is a deep location adjacent to important structures, such as the tympanic membrane, tympanic ostium of the eustachian tube, and inner ear labyrinth. They are articulated to form a chain for the transmission of sound from the tympanic membrane to the inner ear through the tympanic cavity. Auditory ossicles play an important role in the maintenance of hearing.

An abnormal or interrupted ossicular chain can cause hearing loss and conductive deafness. For all patients with damaged auditory ossicles, ossicular chain reconstruction is required to improve or restore hearing. However, postoperative complications, such as stapes footplate fracture and ossicular chain fusion and adhesion, are common after reconstruction. Therefore, it is necessary to reveal the fine anatomy of the ossicular chain, the relationship between its parts, and their functions.

Conventional medical computed tomography (CT) only offers millimeter-scale resolution, which is not sufficient for clear observation of the ossicular chain structure. Micro-CT is a more sophisticated non-invasive imaging technique that has emerged in recent years. It employs a micro-focused X-ray source and offers micron (mm)-scale resolution. This technique can clearly reveal the microstructure of the sample without any destruction, and collect and process several Gbytes of data. Micro-CT has been widely used in many fields, such as orthopedics and stomatology. It has also been applied to the analysis of the microstructure of diseased auditory ossicles

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and auditory ossicles in guinea pigs and other animals (Ritman, 2011; Perilli *et al*., 2012; Buytaert *et al*., 2014). However, there is no report on the study of the microstructure of auditory ossicles in human term fetuses. In this study, micro-CT was used for three-dimensional imaging of the ossicular chain in human term fetuses to analyze the morphological characteristics of the ossicular chain structure, and to measure the structural parameters of trabeculae in incus and malleus. The results provide essential intuitive anatomical data for investigating the reconstruction procedure in tympanoplasty and elucidating the pathological mechanisms of hearing loss. It also presents a new method for observing tiny bones in humans (Park *et al*., 2004; Zou *et al*., 2015; De Greef *et al*.,2015).

## MATERIAL AND METHOD

**Materials.** Four complete ossicular chains from two dead term fetuses in uterus (body donation agreement was signed by the family) were provided by the Department of Anatomy, Inner Mongolia Medical University. Bone samples were collected after degreasing and drying.

**Methods.** The obtained auditory ossicles samples were placed on the stage and scanned by a micro-CT system (Siemens Inveon MM PET/CT, Siemens AG, Munich, Germany) after filtering and air calibration. The scan was performed in high resolution mode using the optimal Siemens Inveon MM PET/CT Bin1 & High scanning protocol. The micro-CT system was uniformly set at 80 kV, 500 mA, and high magnification. Cross-sectional images of the auditory ossicles were obtained at a scanning resolution of 40 mm, layer spacing of approximately 16.7 mm, and layer thickness of approximately 16.7 mm, with a scanning field of 1024 pixels. The image data were imported into the Inveon Research Workplace in a DICOM format. The entire trabeculae were selected as the region of interest in the central ossification area in the cross-sectional images. The parameters of trabeculae were calculated using the build-in multimodal 3D visualization software.

**Statistical analysis.** Data were entered into Excel and SPSS 17.0 for statistical analysis. Data were expressed as mean ± standard deviation (`x ± s). Unpaired samples were tested with an independent sample t test. An a = 0.05 (P < 0.05) was considered significant.

**Micro-CT.** Scan images of normal fetal auditory ossicles were obtained (Fig. 1). The fine anatomy of each auditory ossicles was clearly visible in the images. The marrow cavity of the normal incus had a small size, thick cortical bone, and irregular shape, and contains a three-dimensional network of trabeculae orientated in a transverse or oblique direction (Fig. 2). The marrow cavity of the normal malleus had a smaller size and thicker cortical bone, with almost no trabeculae, and was distributed along the body of the malleus and ended at the handle. The stapes was of a typical "stirrup" shape in the micro-CT scan image; no trabeculae was observed; and the footplate was thick and long elliptical, and connected with slender crura on both sides. No nutrient foramen was found on the surface of incus and malleus by layer-by-layer inspection of the cross-sectional, sagittal, and coronal images. Statistical analysis of the structural parameters of trabeculae in the incus and malleus revealed significant differences in BS/BV and Tb.Th (both P < 0.05), but not in other parameters (P > 0.05) (Table I).

## RESULTS AND DISCUSSION

**Micro-CT.** Offers micron-scale resolution, which solves the long-standing problem of imaging of tiny bone tissues in clinical practice. Micro-CT enables detailed visualization of the shape and internal structure of auditory ossicles, allowing multi-angle observations of the anatomical relationship between auditory ossicles. The trabeculae in the cancellous bone play a general role in supporting hematopoietic tissue in the human body. Moreover, the trabecular orientation is consistent with stress direction. Following Wolf's law, the trabeculae can offer support, reduce and buffer against stress, contain bone marrow, and accommodate for deformation. The auditory ossicles are the smallest and lightest bone in the human body. In this study, micro-CT revealed marrow cavities and trabeculae in the incus and malleus of normal babies. However, the cavity space was small in both auditory ossicles due to the thick cortical bone. Thus, it is conceivable that the trabecular orientation of the incus and malleus aligns with the direction of acoustic stress over the auditory ossicles. In addition, the absence of trabeculae in the stapes indicates high acoustic compliance. No nutrient foramen was observed on the auditory ossicles on micro-CT images, implying that the nutrient may be supplied by the lymph in the inner ear (Peyrin, 2011; Aernouts *et al*., 2012; Tassani & Perilli, 2013; De Greef

Table I. Volume fractions of trabeculae in the marrow cavity of incus and malleus

| | BV/TV (%) | BS/BV ($mm^{-1}$) | Tb.Th (mm) | Tb.N ($mm^{-1}$) | Tb.Sp (mm) | Tb.Pf ($mm^{-1}$) |
|---|---|---|---|---|---|---|
| Incus | 0.51 ± 0.09 | 22.87 ± 5.03 | 0.24 ± 0.29 | 5.74 ± 1.24 | 0.24 ± 0.27 | 4.95 ± 2.12 |
| Malleus | 0.44 ± 0.13 | 35.4 ± 1.76* | 0.06 ± 0.005* | 7.78 ± 2.54 | 0.08 ± 0.04 | 13.47 ± 10.00 |

P < 0.05 compared with incus.

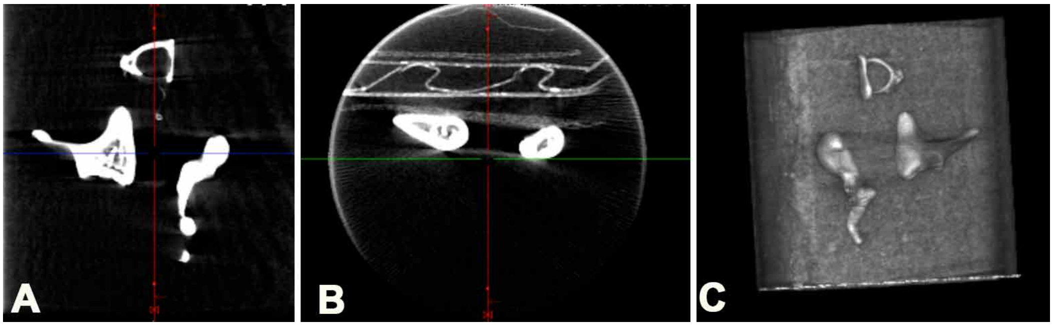


Fig.1. Micro-CT scan images of normal infanthttp://dict.youdao.com/javascript:void(0); auditory ossicles (A horizontal plane, B coronal plane, C three-dimensional images)

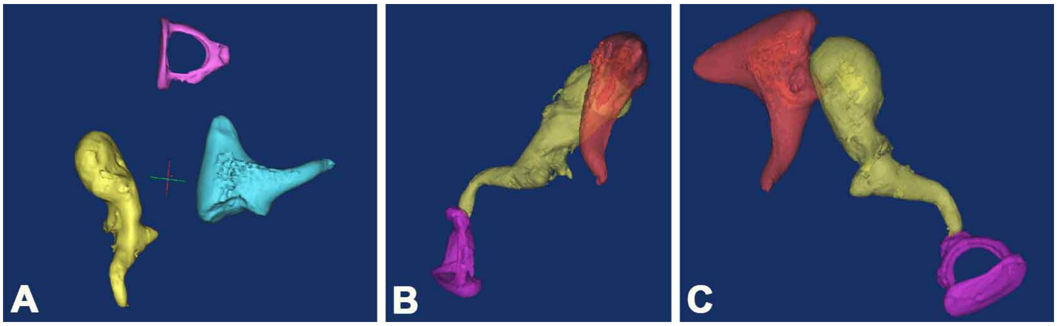


Fig. 2. Three-Dimensional Images of normal infant auditory ossicles for auditory ossicles reconstruction (A Three-Dimensional Images, B And C sagittal plane)

*et al*., 2014; Quam *et al*., 2014; Lee *et al*., 2015; Kauppinen *et al*., 2018).

Otitis media with cholesteatoma is a common otology disease. Cholesteatoma caused by the invagination or perforation of pars flaccida of the tympanic membrane results in accumulated damage to the auditory ossicles, with a damage rate of approximately 90 %. The malleus is first damaged, followed by the incus. The most common finding is bone resorption and destruction of the body of incus and the head of malleus, while the stapes is rarely affected. Bone volume fraction calculated by reconstruction of trabeculae from micro-CT images is an important index for bone quality evaluation of the auditory ossicles. Volume fractions of trabeculae, which represent the bone strength, can be easily calculated by the special software that comes with micro-CT. Park *et al*. used micro-CT for scanning and 3D reconstruction of normal adult auditory ossicles and auditory ossicles damaged by cholesteatoma, and compared their volume fractions. Different volume fractions were measured for auditory ossicles damaged by different types of cholesteatoma. In normal auditory ossicles, lower volume fractions were measured near the articular surface and at the handle of malleus, which were susceptible to infection and trauma (Boutroy *et al*., 2005; Aernouts *et al*.; De Greef *et al*., 2014; Quam *et al*.; Lee *et al*.). Similar results were obtained in this study. Statistical analysis of the selected structural parameters of trabeculae in the regions of interest in the incus and malleus based on the bone volume fraction calculated by reconstruction of trabeculae from micro-CT images of babies revealed significant differences in BS/BV and Tb.Th (both $P < 0.05$). It indicates that the incus and malleus are the first to be damaged during ossicular infection in babies, while the stapes is rarely affected due to the absence of trabeculae.

Conductive deafness is a hearing impairment caused by failure of sound transmission to the inner ear due to diseases of the middle ear or other structures. Ossicular chain reconstruction has been used clinically to restore the hearing of patients with conductive deafness. Although this operation offers a certain efficacy, ideal hearing is not obtained. It is because the sound conduction structure reconstructed cannot reach the physiological level due to failure to simulate the detailed anatomical structure of the middle ear. A too long reconstructed ossicular chain can result in too much tension and penetrate the tympanic membrane, while a too short one can cause dislocation. Micro-CT can accurately measure the size of each anatomical structure of the auditory ossicles, and analyze the 3D shape of the ossicular chain from multiple angles and multi-planes through 3D reconstruction. This facilitates the positioning of the auditory ossicles, guides the effective and reasonable articulation of ossicular replacement prosthesis, and improves the movement of the tympanic membrane and ossicular chain (Cunningham & Black, 2013; Fallon *et al*., 2014; Hutchinson *et al*., 2017; Zdilla *et al*., 2018).

In future research, a microscopic 3D finite element model created from micro-CT images and digital 3D printing can be used for sonodynamic calculation and analysis of normal auditory ossicles and ossicular replacement prosthesis of different materials, in order to investigate the factors affecting hearing restored in patients with conductive deafness (Gentil *et al*., 2014).

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**RESUMEN:** Este estudio tuvo como objetivo investigar la morfología microanatómica de la cadena osicular en el feto a término con micro-CT, con el fin de analizar los parámetros de la estructura osicular interna que pueden afectar la conducción del sonido. Cuatro cadenas osiculares de dos fetos a término fueron examinadas por micro-CT. Se calcularon y compararon los parámetros estructurales relacionados de las trabéculas dentro de los incus y malleus. Se analizó la estructura anatómica fina de los osículos. Se observó claramente la microestructura de cada osículo en los fetos y la cavidad medular en el incus y el malleus. En el análisis estadístico de los parámetros estructurales de las trabéculas en el incus y el malleus, se encontraron diferencias significativas en BS / BV y Tb.Th (P <0,05). Micro-CT permite la visualización de la estructura osicular interna. Los osículos en el feto a término tienen buena calidad ósea. Los datos obtenidos de la estructura ósea ayudarán para aclarar las funciones fisiológicas de los osículos auditivos fetales normales.

**PALABRAS CLAVE: Osículos auditivos; Trabecula; Micro-CT.**

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