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쥐 와우의 영상 분석에 있어 Micro-CT의 가능성과 한계

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ABSTRACT

Possibility and Limitation of Micro-CT in Cochlear Imaging of Rat

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The objective of this study is to investigate the degree of visualization for the macro-, micro-, and nano-structures of the cochlea with x-ray micro-CT based on a cone-beam geometry for rats. This study will provide a basic criterion to compare the visual images obtained from other techniques. Before obtaining the visual images, the hearing thresholds were obtained with auditory brainstem responses (ABR). A x-ray micro CT based on a cone-beam geometry for a small animal was used for examining the macro-, micro-, and nano-structures of the cochlea in rats. X-ray micro CT provided a clear depiction of the basal, middle, and apical cochlear turns. X-ray micro CT allows visualizing the macro-structures of the cochlea. However, it did not well depict the micro- and nano-structures of the cochlea. The present study was the first attempt to visualize the macro-structures of the cochlea with x-ray micro CT based on a cone-beam geometry for rats. To visualize the micro- and nano-structures of the cochlea, optimal parameter values of x-ray micro CT should be found and many factors such as signal-to-noise ratio, resolution, image averaging, pixel, pixel number, pixel value, exposure time, and binning should be properly considered.

KEY WORDS : X-ray micro CT, Macro-, micro-, and nano-structures, Cochlear mechanics, Visual image, Cone-beam geometry

INTRODUCTION

After the clinical computed tomography (CT) was first developed in 1967 by G. Hounsfield, x-ray CT has been widely used for diagnosis in biomedical fields. X-ray CT is a medical imaging method using tomography created by digital geometry processing to generate a three dimensional image of the inside of an object. The use of x-ray CT has increased dramatically over the last two decades in many countries and has been applied to various organs such as brain, lungs, pulmonary angiogram, cardiac, abdominal and pelvic, and extremities. The popularity of x-ray CT is based on several advantages over traditional radiography: (1) the ability to eliminate the superimposition of images of structures outside the area of interest, (2) the inherent high-contrast resolution, and (3) the multiplanar reformatted imaging.

The use of x-ray micro CT has been extended to the ear consisting of the outer, middle, and inner ears. Out of three sections of the ear, the middle and inner ears have been the main concern for x-ray micro CT because they are very difficult to visualize. Normal and abnormal mechanics of the middle ear could be well depicted by x-ray micro CT (Lane et al., 2006). Particularly, ossicular erosions in the clinical cases of cholesteatoma, traumatic dislocation, and congenital anomalies of the ossicular chain and oval window could be easily diagnosed.

The cochlea of the inner ear is the most important organ

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of hearing. The cochlea consists of the macro-, micro-, and nano structures. The macro-structures include scala vestibule, scala media, and scala tympani. The micro-structures of the cochlea include basilar membrane, tectorial membrane, and stereocilia while the nano-structures include the outer and inner hair cells which are related with two transduction processes: mechano-electrical transduction and electro-mechanical transduction (Choi, 2010, 2011a; Choi et al., 2010). The main reason that x-ray micro CT has been used in audiology and otology is that it allows non-invasive visualization for audiologic/otologic diagnosis. In addition, x-ray micro CT has been used for evaluation of visualization of the electrode in the scala tympani, the insertion depth and positioning of the contacts, the electrode status after insertion, and damage to the basilar membrane and to the lateral and medial cochlear walls (Postnov et al., 2006).

Although x-ray micro CT provides a clear visualization of the middle ear, we are not sure of whether the macro-, micro-, and nano-structures of the cochlea can be visualized with x-ray micro CT. Therefore, the objective of this study is to investigate the degree of visualization for the macro-, micro-, and nano-structures of the cochlea with x-ray micro-CT based on a cone-beam geometry for rats. This study will provide a basic criterion to compare the visual images obtained from other techniques.

MATERIALS AND METHODS

1. Animal preparation

Three rats (Rattus norvegicus) weighting 200-500 g were used in this study. The experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committees of the Catholic University of Daegu. Before euthanization, the hearing threshold of the animals for both ears was measured by auditory brainstem response (ABR) recorded under light ketamine and xylazine anesthesia and small supplemental doses (1/3 of initial dose) if needed using an active needle electrode and a reference electrode placed proximal to the test ear and the non-test ear, respectively and a ground electrode placed at the vertex. Acoustic stimuli consisting of tone pips at frequencies of 0.5, 1, 2, 4, 6, 8 kHz were generated by a computer-aided system (Intelligent Hearing Systems, Miami, FL) and transduced through the computer controlled attenuator to a 3A insert earphone. The electrical responses were amplified (x 100,000), filtered (100–3,000 Hz), digitalized, and averaged at a sample rate of 1024 for each level. The hearing thresholds were determined by 10-dB descending steps and 5-dB ascending steps and defined as the midpoint between the lowest level of a clear response and the next level where no response was existed (Choi, 2011b, 2011c; Floyd et al., 2008). The hearing thresholds of the animals used in this study were within normal range below 30 dB SPL across the whole frequency range.

The rats were humanely euthanized with transcardial injection of sodium pentobarbital under anesthesia with a mixture of ketamine (20 mg/kg) and xylazine (1 mg/kg). Both ears of each animal were used for visual imaging (Choi et al., 2008, 2011). After the temporal bone was immediately removed from the skull, the cochleae were perfused from the oval window or the round window to a hole made at the apex with a solution of 4% paraformaldehyde in phosphate buffer for 24 h at 4^{o} C. The cochlear tissues were dissected and washed with PBS. The cochleae were mounted on a glass slide as surface preparations and examined for a micro-CT.

2. Micro-CT system

The entire experiments for visual images with x-ray micro CT were performed at Wonkwang University. X-ray micro-CT based on a cone-beam geometry for a small animal was used for examining cochlear image of rats (윤권하, 2009). The micro-CT was mainly composed of an x-ray tube with tungsten target, a sample holder made from plastic of 2 mm thick, and a digital detector of CMOS (Complementary Metal Oxide Semiconductor) (Fig. 1).

The detector had sensing area of 123 mm × 112 mm and the pixel size was 100 μm × 100 μm. The micro-CT was a gentry-rotation type in which the x-ray tube and the detector simultaneously rotate around the rotation center and a sample of the cochlear tissue was fixed on the rotation center. Projection magnifications (high, middle, and low modes) under the cone-beam geometry were made by controlling the distance between the x-ray tube and the detector from the rotation center. The maximum rat size to be examined was

50 mm in diameter at the low magnification mode. In the high magnification mode with 3.5X in which source-to-object and source-to-detector distances were 62.85 mm and 220 mm, the spatial resolution was 35 μm.

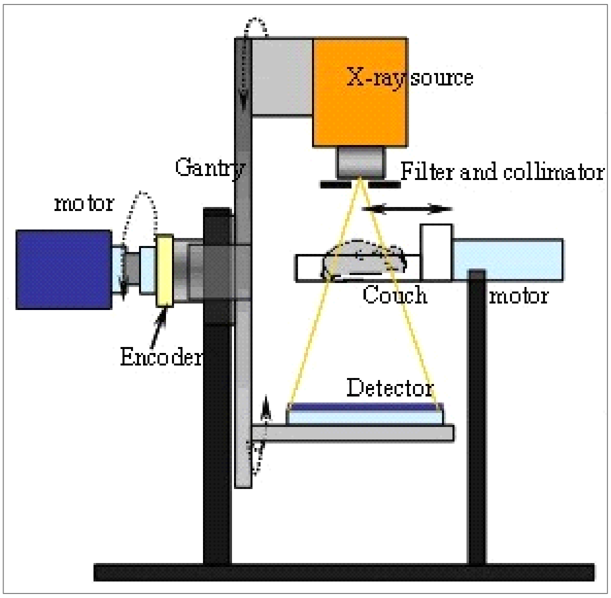

Figure 1. Illustration of experimental setup for visual images

In examining the cochlear image, the tube voltage and current were 70 kV and 90 μA, respectively and the exposure time for obtaining a projection view was 34 ms. The total number of image was 720, one image per 0.5 degree, and 1024 × 1024 × 512 slice images were obtained by the cone-beam reconstruction based on Feldkamp algorithm. For documentation, the resulting data were saved to a stack of TIFF images. A three-dimensional tomography image was generated by volume rendering.

RESULTS

The micro-CT allowed visualizing the structures of the cochlea. Although three rats were used as subjects, all results were similar to each other. The best images were chosen and presented for analysis. Fig. 2 shows the cochlear duct coiling from the base to the apex. The height of the cochlear turns ranges from 0.78 to 0.56 mm. When the cochlear turn reaches to the apex, the cochlea duct becomes narrower. Other figures show each cochlear turn from the

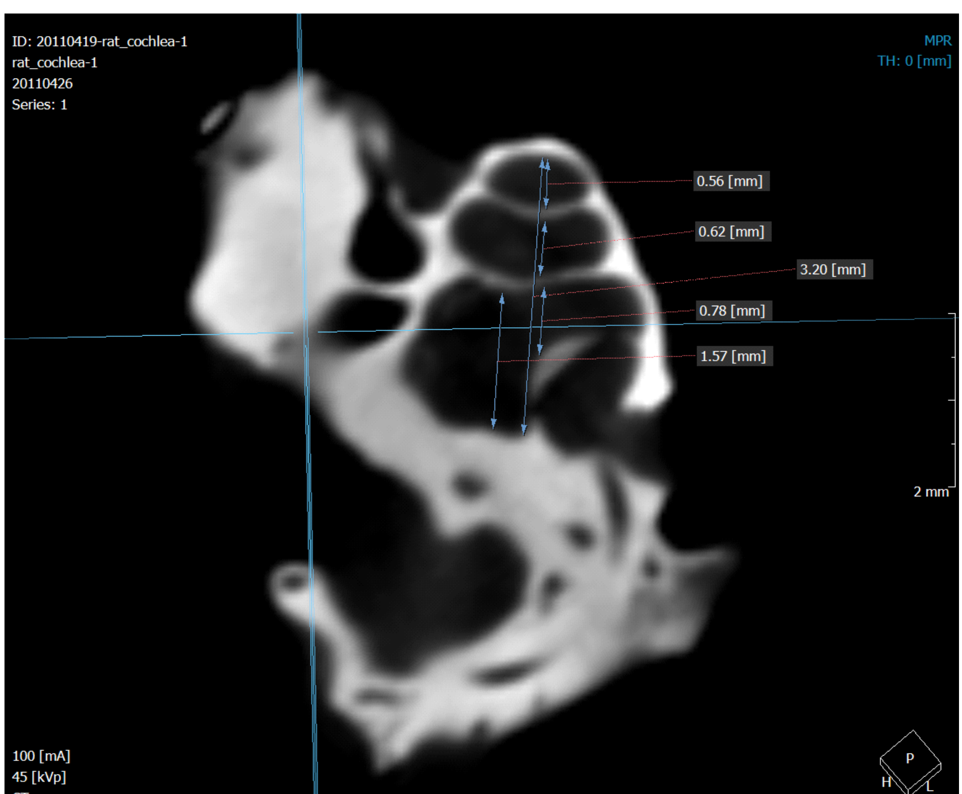

Figure 2. A micro-CT image at whole cochlea. Each turn shows its height

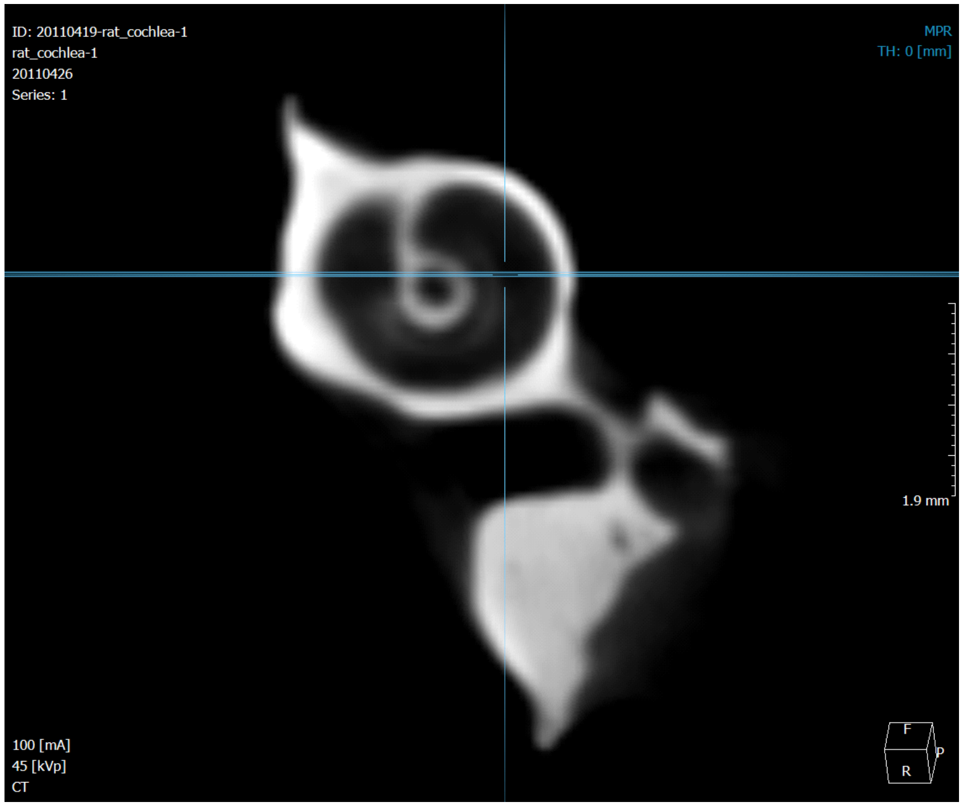

Figure 3. A micro-CT image at the basal cochlear turn

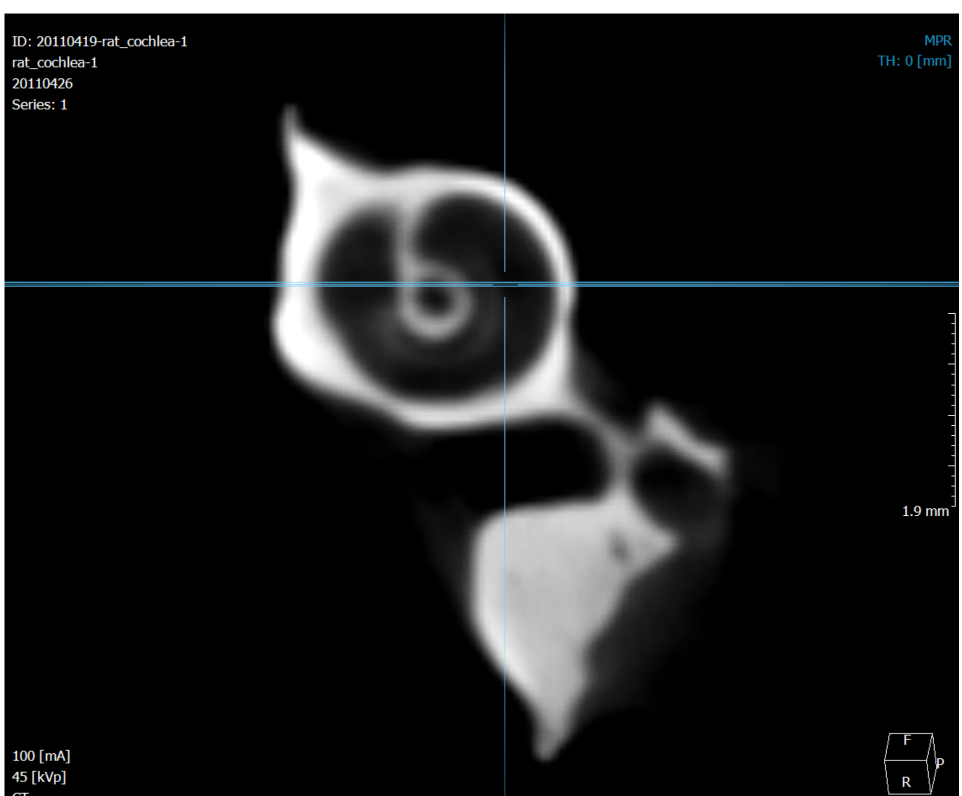

Figure 4. A micro-CT image at the middle cochlear turn

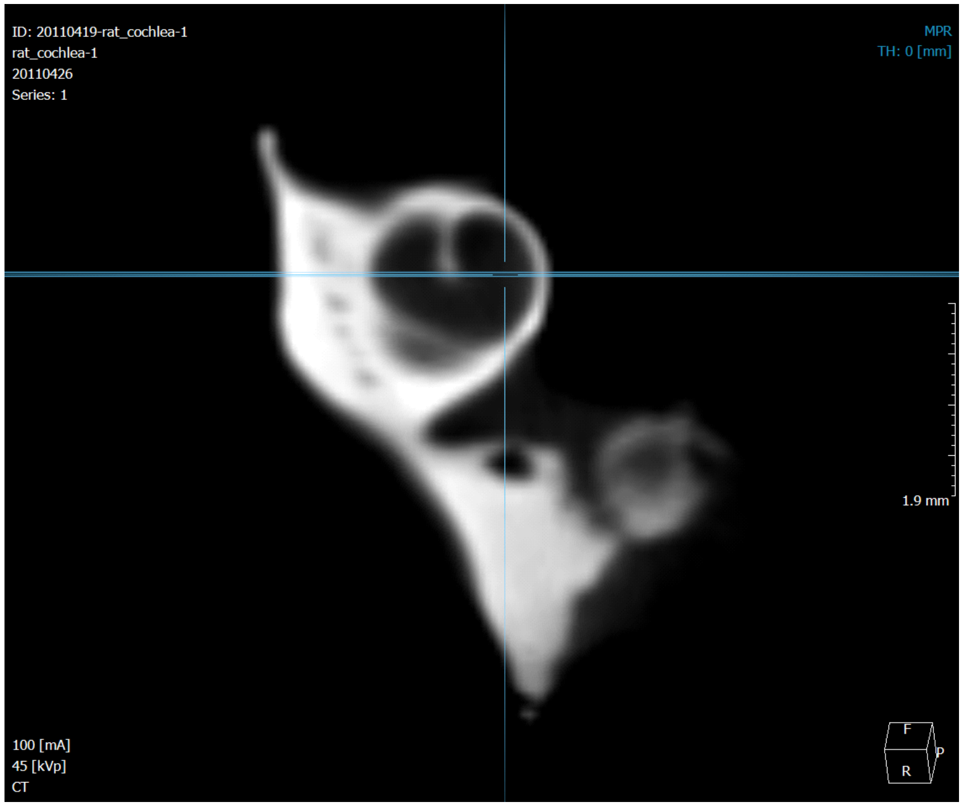

Figure 5. A micro-CT image at the apical cochlear turn

base to the apex (Fig. 3-5). Fig. 3 illustrates the basal cochlear turn. Fig. 4 shows the middle cochlear turn while Fig. 5 illustrates the apical cochlear turn.

DISCUSSIONS AND CONCLUSIONS

The present study is the first attempt to obtain visual images of the cochlea with a micro CT based on a cone-beam geometry for rats. Clinically, the micro-CT provides several advantages for audiological or otological diagnosis. It allows detecting the sites of lesion or presumed abnormality for different degrees and types of hearing loss as well as detecting abnormalities affecting the middle ear, called conductive hearing loss (Ali et al., 1993; Lane et al., 2006). The current study did not include visual images of the middle ear because the middle ear structure is not our main concern. Visual images of the cochlea obtained from this study are consistent with the macro-cochlear images obtained from multidetector CT (Lane et al., 2006; Tomandl et al., 2000). The macro-structures of the cochlea including scala vestibule, scala media, and scala tympani were well shown but the micro-cochlear images including the organ of Corti, basilar membrane, and tectorial membrane were faintly presented.

On the other hand, the use of the micro CT in otology and audiology is involved with cochlear implant. The micro CT has been used as innovatory tool for in vitro visualization of the inner ear tissues as well as for evaluation of the electrode damage and/or surgical damage during implantation of the cochlear implant electrodes (Postnov et al., 2006; Whiting et al., 2007). Another major advantage of micro CT is its non-invasiveness which can simultaneously image both bones and soft tissues (Poznyakovskiy et al., 2008). For the purpose of audiologic diagnosis, the micro-CT should be used with other audiologic measurements which measure the functions of the outer, middle, and inner ears as well as the brain. Based on the relationship between visual images of the cochlea and the results of the functional tests of the cochlea, the effects of the micro-CT could be maximized with the audiologic measurements including pure-tone audiometry, speech audiometry, acoustic immittance measurements, ABR, auditory evoked potentials, otoacoustic emissions, and other tests. The micro-CT can be also used with magnetic resonance imaging (MRI) in many cases of the brain injury. The micro-CT is preferable in evaluation of the temporal bones, specially paranasal sinuses, central nerve system infections, acute trauma, and cholesteatoma while MRI is preferable in evaluation of the skull base, pituitary gland and parasellar regions, and the internal auditory canals (Rose, 1996).

Recently, although many techniques for the research of cochlear mechanics have been developed, cochlear processes cannot be investigated completely with existing measurement methods such as a cone-beam geometric model and interactive direct volume rendering. The current image technologies do not provide complete investigation of the micro-and nano-structures of the inner ear. The nano-structure of the cochlea including the mechanisms of active process and amplification in cochlea originating from outer hair cells is the most important organ of hearing (Choi, 2010). The active process indicates the presence of a cochlear amplifier to improve hearing sensitivity at low sound intensity levels. Within outer hair cells, the cochlear amplifier is directly involved in two transduction processes: mechano-electrical transduction and electro-mechanical transduction (Choi et al., 2010; Choi, 2010, 2011a). This is the biophysical property that differenciates outer hair cells from other known cell types. The nano-structure of the cochlea can be better visualized with a X-ray nano-CT in future. In addition, the CT contrast agents such as iodine, bismuth, gold, or gadolinium nanoparticles can be specifically used for visualization. Recently, interior tomography has been developed to overcome the long-standing barrier to realize the full-potential of X-ray nano-CT (Wang & Yu, 2010). The combination of X-ray nano-CT and interior tomography will provide a versatile nano-imaging tool that can visualize fine features within a larger object and use a much lower radiation dose and in much less time (Wang & Yu, 2010). The nano-structures of the cochlea can be visualized with 3D X-ray tomography system in the Pohang Light Source (PLS) 7B2 Bio-imaging Beamline, which is a third generation synchrotron radiation facility with the operating energy of 2.5 GeV at Pohang Accelerator Laboratory. The 7B2 Beamline consists of beam shaping slits, a X-ray window, X-ray intensity attenuators, X-ray intensity monitor, and X-ray imaging optics housed in the hutch. Now we are collecting imaging data of the cochlea with 3D X-ray tomography system to compare those obtained from the micro-CT based on the cone-beam geometry.

The visual images of the micro-CT can be affected by many factors such as signal-to-noise-ratio, resolution, image averaging, pixel, pixel number, pixel value, exposure time, and binning. For example, signal-to-noise ratio (SNR) of the micro-CT can be changed by the parameters such as filter use, exposure time, image averaging, binning mode, and X-ray beam control. When the exposure time is longer, the time to obtain a visual image slice is longer and SNR is higher. When the binning increases from 1x1 to 2 x 2 (in other words, the number of pixels decreases), SNR is improved while the resolution is worse. When the image averaging increases, SNR is better and it takes more time. In addition, a commercial software program, called Amira can be used to reconstruct geometrical 3-D model of the cochlea. With this program, it is possible to create geometric models from stacks of TIFF images and transform them to VRML files. For this process, it is very important to have a skilled professional for high quality 3-D reconstruction.

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