Analysis of Mouse Cochlea in Decalcification Effects of Ethylenediamine Tetraacetic Acid Liquid Using Optical Coherence Tomography

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ABSTRACT

To study internal structure in the pathology of cochlea many research groups often used methods of decalcifications. There are several means of decalcifications, especially we used ethylenediamine tetraacetic acid (EDTA) liquid to enhance the image of cochlea of mouse using optical coherence tomography (OCT). But analysis of decalcifications of EDTA liquid to image of cochlea of mouse using OCT has a little research. So, here we show the specific information of decalcifications of EDTA liquid in internal imaging of cochlea of mouse using method of image processing. In this study, the decalcification of cochlea of mouse has the best effects when soaking in EDTA for 7 days.

KEYWORDS

Cochlea, decalcification, OCT, EDTA, image analysis.

1 Introduction

Inner structure of mouse cochlea is closely involved with errors of listening function (1). Therefore, like this study is important. To study of inner structures of cochlea usually used histological methods (2). But, there is a weakness that a sample of cochlea be damaged using the method. So, many research groups use an optical coherence tomography (OCT) (3). OCT have a high resolution about the number of micrometer unit and noninvasive modality (4, 5). Nonetheless, OCT also have some problem is that if beam out of OCT can't penetrate inner of sample, we can't see image of inner structure. When we take an inner image of cochlea using OCT, beam can't penetrate because of outside thick bones of the cochlea (6). That is a limitation of OCT. But we tried to overcome the limitation using a decalcification method. Decalcification has an effect on removing calcium of hard tissue like a bones and making a transparent and lighter (7). Therefore, beams of OCT more deeply penetrate inner structure of sample hence, the image of OCT have a more deep and bright information. In the like this method of decalcification, there are acid decalcification using 10% Formic acid, methods using a Paraffin oven, DECAL, RHS-1 and etc. But like this methods of decalcification have not much research in imaging using OCT (8). Doing a histology, EDTA liquid usually be used to image using OCT and Akinobu Kakigi et al. also researched like this. Nevertheless, until now quantitative information of EDTA periods and effects are insufficient (9). So, in this study, we did decalcify cochlea of mouse then took an image of OCT by the period of decalcifications. And we will offer some information of most beneficial EDTA period and the effect when taking an image of mouse cochlea using OCT.

2 Methods and Materials

2.1 Mouse cochlea sample preparation

A total of 5 cochlea of ICR-Mouse male (living of 5 weeks) with weighing from 27 g to 29 g were used. Before do the surgery, we fed for a week to stabilize the mouse after arriving first time at laboratory. We did cardiac perfusion before taking a cochlea. After cardiac perfusion,

we took away the cochlea from mouse. In cardiac perfusion, we injected 30 mL of a PBS pH7.4, 1X after that, injected 30 mL of 4% Paraformaldehyde, 1X PBS using syringe. Cochlea is stored on cryo tube of 1.8 mL for 1 day in 4% Paraformaldehyde, 1X PBS at room temperature. And after taking a control image of cochlea using OCT, soak on cryo tube of 1.8 mL in EDTA 10% pH7.4. And cochlea of both animals is kept on shaker with shaking 80 times/min at room temperature. A reason of keeping on shaker is to more decalcify inner of the cochlea. Keeping on shaker we checked a period of EDTA to adjust the timing of taking OCT. We changed the EDTA every two days for keeping on speed of decalcifying of the sample.

2.2 OCT system configuration

We used OCT equipment of 1300 nm swept source OCT. The system have a 1300 nm of center wavelength, about 97 nm of spectral bandwidth at -10 dB cut off point, 100 kHz of axial scan rate. And the system have a 10 mm of the maximum imaging width, approximately 12 mm of the maximum imaging depth, 25 μm of transverse resolution and about 16 μm of axial resolution in air.

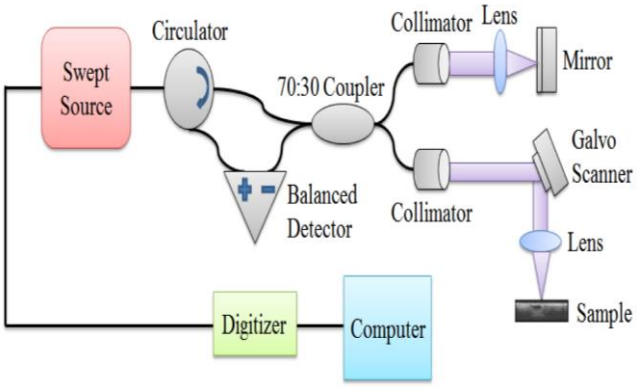

Fig. 1 The schematic of swept-source OCT system.

The imaging dates of OCT have taken step before immersed in EDTA solution and after immersed in EDTA solution that 1 day, 3 days, 7 days, 14 days, 21 days about cochlea of mouse. When taking an image of OCT, we compared to previous image and finely moved a stage to take same position by distinguishing part. Size of image has 1034 pixel about x-axis and 610 pixels about y-axis in the system. When taking a B-scan image, field of view of mouse are 4 mm of x-axis and 6 mm of x-axis. We analyzed cochlea image of OCT using Matlab program. Loaded image be averaged on lateral direction of intensity of each pixel and then the image be showed through graph of A-scan profile. Also, we applied this method about the sample of cochlea from control to 21 days in EDTA. We compared the degree of improving about contrast and penetrated depth of cochlea image of sample according to period of EDTA.

3 Results and Discussion

Using our SS-OCT system, we performed the mouse cochlea experiment, which was soaked in EDTA for the decalcification. The image acquisition was carried out on the 1st, 3rd, 7th, 14th, and 21st days, respectively. The obtained corresponding images are shown in Fig. 2. The cross-sectional image of the control sample is shown in Fig. 2(b-c) depicting the least depth visibility comparatively. An identifiable depth visibility can be notified in the remaining images according to the continuation of the process. Therefore, it can be precisely analyzed that the declassification based optical clearing method plays vital role for the attainment of micro-structural depth information.

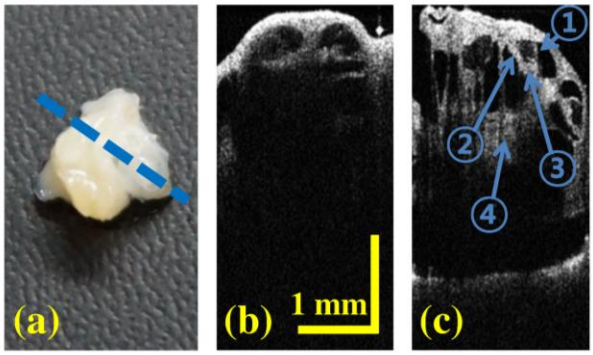

Fig. 2 Image of mouse cochlea: (a) photographs image of mouse cochlea, (b) Control sample OCT image, (c) 7th OCT image. ①: Reissner's membrane, ②: Basilar membrane, ③: organ of Corti, ④: modiolus.

The averaged intensity is indicated in Fig. 3. Intensity quantification could be notified in all the experimental plots within the depth range of 2 - 4 mm. Compared to the control sample, increase of the intensity can be indicated in all the other plots beyond the depth range, which verifies the decalcification effect due to EDTA. Subsequently, we evaluated the total intensity fluctuation of the entire image according to the period of decalcification.

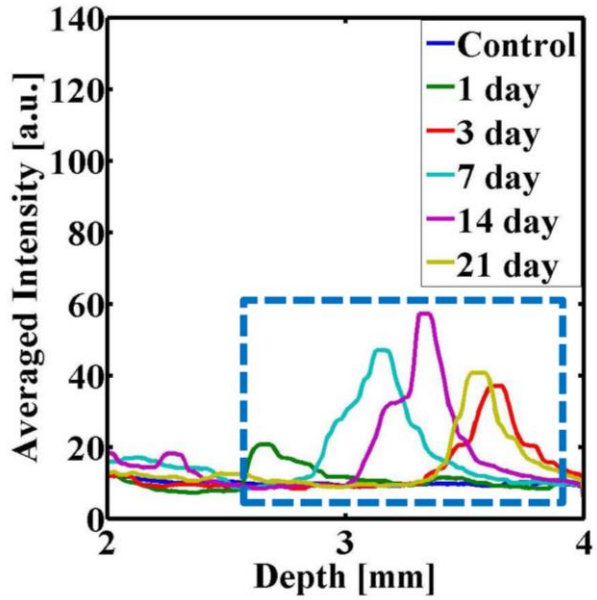

Fig. 3 Intensity of decalcified mouse of cochlea OCT image in depth direction on each specific day.

The total intensity fluctuation is illustrated in Fig. 4 significant increase of the total intensity can be confirmed as expected in the decalcified samples compared to the control sample. Note that the maximum intensity can be recognized on the 7th day, and further, the total intensity of the remaining days is confirmed to be saturated. Therefore, it was revealed that the most optimal clearing days of decalcification for mouse cochlea was considered as 7 days through the acquisition of the image with the maximum intensity.

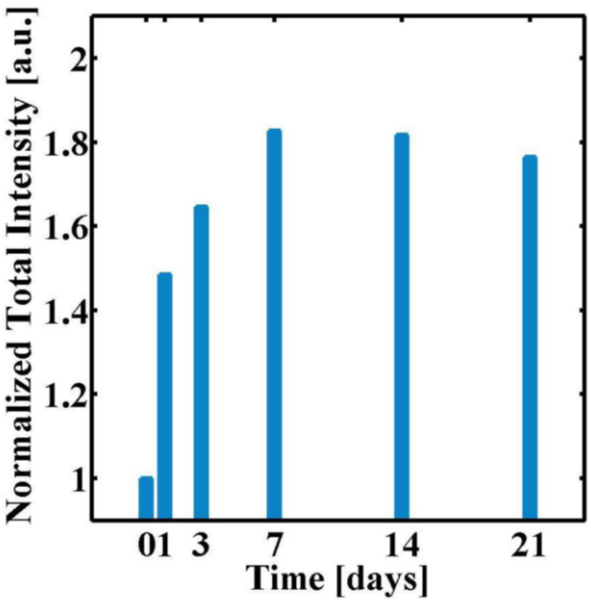

Fig. 4 Intensity analysis in depth direction on each specific day.

4 Conclusions

Analyzed results of cochlear OCT image successfully illustrated the capability of the proposed method by providing clearly visible micro-structures in the depth direction and by clarifying the challenging experimental limitation of imaging micro-structures. The effectiveness of this proposed optical clearing method for mouse cochlea was identified optimal results were identified after 7 days.

5 TABLES and FIGURES

Fig 1. The schematic of swept-source OCT system.

Fig 2. Image of mouse cochlea: (a) photographs image of mouse cochlea, (b) Control sample OCT image, (c) 7th OCT image. ①: Reissner's membrane, ②: Basilar membrane, ③: organ of Corti, ④: modiolus.

Fig 3. Intensity of decalcified mouse of cochlea OCT image in depth direction on each specific day.

Fig 4. Intensity analysis in depth direction on each specific day.

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