Quantitative imaging of cochlear soft tissues in wild-type and hearing-impaired transgenic mice by spectral domain optical coherence tomography

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Abstract: Human hearing loss often occurs as a result of damage or malformations to the functional soft tissues within the cochlea, but these changes are not appreciable with current medical imaging modalities. We sought to determine whether optical coherence tomography (OCT) could assess the soft tissue structures relevant to hearing using mouse models. We imaged excised cochleae with an altered tectorial membrane and during normal development. The soft tissue structures and expected anatomical variations were visible using OCT, and quantitative measurements confirmed the ability to detect critical changes relevant to hearing.

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1. Introduction

The auditory system serves to amplify and convert sound pressure waves into neuronal signals. Sounds waves are first collected and funneled by the external ear to the tympanic membrane. These vibrations are then transferred through the middle ear ossicles to the oval window of the cochlea, the hearing organ of the auditory system. The cochlea is shown in Fig. 1A. It is spiral shaped and encased in bone, but the key intra-cochlear structures that convert mechanical motion to electrical signals are composed of soft tissue, shown in Fig. 1B. More specifically, the inner (IHCs) and outer hair cells (OHCs) sit atop supporting cells and the basilar membrane (BM) and perform mechano-electrical transduction. The stereocilia of the OHCs connect to the tectorial membrane (TM), which is in turn connected to the spiral limbus. The IHCs relay the afferent signals to the brain via auditory neurons (AN), which are housed in the central, bony modiolus. Reissner's membrane (RM) serves as a diffusion barrier to separate the fluids within scala media (SM) from that of scala vestibuli (SV). The spiral ligament (SL) contains the stria vascularis that maintains the ionic gradients in the SM necessary for normal hearing. Damage to any of these structures results in sensorineural hearing loss.

![Fig. 1. (A) The cochlear has three chambers, scala vestibuli (SV), scala media (SM), and scala tympani (ST). The auditory neurons (AN) sit within the central core, the modiolus. The structure within the box is expanded in (B). (B) The organ of Corti contains three rows of outer hair cells (OHCs) and one row of inner hair cells (IHCs). The hair cells sit along with supporting cells on the basilar membrane (BM), which is fixed at the spiral limbus and osseous spiral lamina (OSL) medially and the spiral ligament (SL) laterally. Hair cell stereocilia are deflected when shearing forces develop between the apical surface of the hair cells and the tectorial membrane (TM) during sound transduction. Auditory nerve fibers (AN) connect the hair cells with the brainstem.](image)

In a human, the cochlea is about 1 cm in diameter, yet the soft tissues range on the order of 10 to 100 µm in thickness. As such, current clinical imaging modalities such as magnetic resonance imaging (MRI) and computed tomography (CT), which have resolutions of approximately 1 and 0.5 mm respectively [1,2], simply do not provide the necessary resolution required to detect disturbances in the intra-cochlear soft tissues associated with hearing loss. This is illustrated in Fig. 2. Figure 2A (left) demonstrates a CT image of a cochlea (arrow) in a deaf child that was read as normal. Although audiometric tests revealed that the cochlea was malfunctioning, the physical basis for the hearing loss was presumably too small to be appreciated by the imaging technique. CT can, however, detect gross malformations. For example, Fig. 2A (right) shows a CT image of a grossly malformed cochlea (arrow) in another deaf child in which there is a complete lack of bone within the modiolus of the cochlea. Similar problems exist with MRI. Figure 2B (left) shows an MRI image of a cochlea in a deaf child that was also read as normal, whereas Fig. 2B (right) shows a cochlea that is about half the size of a normal cochlea (arrow).
Fig. 2. (A) CT and (B) MRI images of four different deaf patients. On the left are cochleae (arrows) that appear normal, but are likely to have important anatomic changes if post-mortem histopathologic studies were performed. On the right are examples of the types of gross malformations that can be detected with the latest imaging techniques (arrows).

As seen, current clinical imaging methodologies only allow for the detection of gross bony malformations. However, post-mortem histological analyses of human temporal bones reveal that the most common causes of hearing loss, i.e. age-related, noise-induced, ototoxic exposure, and genetic mutations, only produce changes in the intra-cochlear soft tissues. These changes can include hair cell loss, TM malformation or separation from the OHCs, loss of AN, atrophy of the stria vascularis, and/or loss of auditory neurons [3]. Since most forms of hearing loss do not have any appreciable findings on CT or MRI [4], this dramatically limits the ability to understand and treat hearing loss in individual patients.

There is a need for better cochlear imaging technology to help clinicians and researchers visualize the cochlea at a higher resolution. Therefore, we sought to apply the technique of optical coherence tomography (OCT) to this problem. OCT is a noninvasive imaging technique with micron scale resolution that allows for 3-dimensional imaging within scattering media [5]. We chose to use spectral (or Fourier) domain OCT as it provides a higher signal-to-noise ratio and faster imaging speeds compared to time domain OCT [6]. OCT is already an established imaging technique for ophthalmologic care [7–9] and is under investigation for intravascular imaging of coronary vessels [10,11]. OCT has other potential clinical applications in the fields of bronchology [12], cancer research [13–15], and gastroenterology [16,17], among others.
To test the efficacy of OCT for cochlear imaging, we turned to a commonly used animal model in auditory research, the mouse. The mouse cochlea functions in a nearly identical fashion to that of the human cochlea [18], and transgenic mice with human hearing loss mutations are available for study. Our objective was to determine whether spectral domain OCT could provide anatomical information about the intra-cochlear soft tissues relevant to hearing in mice. We imaged normal mouse cochleae, as well as cochleae from transgenic mice that have a mutation in the Tecta gene [19], which is based upon a human hearing loss mutation [20]. This mutation causes hearing loss by altering the structure of the TM, the biomechanics of the TM, and by increasing the risk of outer hair cell loss after noise exposure [21,22]. The OCT images were compared to histological images of fixed, sectioned tissue, the standard method to assess cochlear anatomy. Furthermore, we imaged normal cochleae at different developmental ages in order to determine whether growth and development could be assessed. We also analyzed the effect of ossification of the apical otic capsule on image quality at the soft tissues. Preliminary results of this work have been presented [23].

2. Materials and methods

2.1 System description

The spectral domain OCT system is shown in Fig. 3. The source consisted of 140 fs pulses of 950 nm light from a modelocked Ti:sapphire laser (Chameleon, Coherent, Santa Clara, CA). The light was focused into an ultrahigh numerical aperture single mode optical fiber (UHNA3, NuFern, East Granby, CN) in order to broaden the spectral bandwidth [24,25]. Launching 250 mW into the fiber resulted in spectral broadening of the source to a full width at half maximum of ~80 nm, resulting in a theoretical axial resolution of ~5 µm in air or ~4 µm in solution [26]. The light exiting the fiber was then collimated and focused into a 2x2 (50:50) fiber-fused coupler (WA10500202B2111-BC1, AC Photonics, Santa Clara, CA). One of the output ports was coupled into the X-Y galvo-mirror scan head of an upright microscope (MOM, Sutter Instruments, Novato, CA), which served as the sample arm; the other was used as the reference arm. The average power incident on the sample tissue surface was ~10 mW.

![Fig. 3. Schematic of the spectral domain OCT system.](image)

The reflected light from both arms was then combined in the fiber coupler. The resulting spectral interferogram was measured using a custom spectrometer based on a high speed line scan camera (AViiVA SM2 CL 2014, E2V, Tarrytown, NY) capable of line rates up to 28 kHz. A camera integration time of 30 µs was used for all images presented herein. The dynamic range of the 12-bit camera was ~70 dB, as referenced to the standard deviation of the dark current and read noise. In custom software written in MATLAB (MathWorks, Natick,
MA), the interferogram was transformed into k-space, and the magnitude of the Fourier transform was computed to produce the depth-resolved sample reflectivity or A-line. The signal-to-noise ratio of the system was ~90 dB, as determined by comparing the A-line peak of a mirrored surface to the standard deviation of a region 500 µm away. Three-dimensional images were created from a series of X-Z slices scanned in the Y direction spaced 5 µm apart, each of which was averaged 4 times unless stated otherwise. The lateral resolution, determined experimentally by imaging microspheres, was ~10 µm. The contrast and intensity curve properties were adjusted in ImageJ or Photoshop CS4 (Adobe, San Jose, CA) to optimize the image. Measurements were, however, made on unaltered images.

2.2 Imaging of specimen preparations

The Stanford University and Baylor College of Medicine Institutional Animal Care and Use Committees approved the study protocols. After sacrifice with an overdose of a ketamine/xylazine mixture, cochleae were isolated from post-natal day 3 (P3), P15, or >P30 (adult) mice. We studied normal-hearing mice (CBA strain) and three genotypes of a transgenic mouse strain that contained a human hearing loss mutation that produces a malformed TM (Tecta<sup>wt</sup> (wild-type), Tecta<sup>C1509G</sup> (heterozygous), and Tecta<sup>C1509G/C1509G</sup> (homozygous) genotypes). Each cochlea was glued upright into a chamber before being imaged. The cochlea was immersed in either an external solution of (in mM) 150 NaCl, 4 KCl, 2 MgCl<sub>2</sub>, 1.5 CaCl<sub>2</sub>, 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), and 10 glucose or phosphate buffered solution (PBS). When indicated in the text, a hole was made in the bone overlaying the region of interest with a fine knife and pick. All images were collected within two hours of animal sacrifice.

2.3 Paraffin-embedded histological sections

Mice were euthanized as previously mentioned. The cochleae were isolated from the temporal bone in PBS and fixed in either 4% paraformaldehyde or a solution containing 60% ethanol, 30% formaldehyde, and 10% glacial acetic acid overnight at 4°C. After a triple wash in PBS, the cochleae were decalcified with 0.5 mM ethylenediaminetetraacetic acid (pH 8.0) for 2 days at room temperature. After another set of PBS washes, they were dehydrated with gradient ethanol and Histo-clear (Electron Microscopy Sciences, Hatfield, PA) and embedded in paraffin. Serial sections of 7 µm thickness were prepared in the mid-modiolar plane and stained with hematoxylin and eosin. Images were taken at either 5X or 10X magnification on a LSM 5 Exciter (Carl Zeiss, Thornwood, NY).

2.4 Image analysis

Measurements were made in ImageJ or Photoshop CS4. No adjustments were made to the images for these purposes. We measured the area of the TM, thickness of the hair cell epithelium, distance between the TM and hair cell epithelium, thickness of the spiral limbus and OSL, thickness of the RM, and thickness of the bone and SL at the RM. We also measured the penetration depth and image intensity at the soft tissues. The structural measurements were made on slices of the image stacks that were from the middle third of the cochlea. As well, we recorded the pixel intensity values across the internal spiral sulcus.

To measure the area of the TM, the outline of the TM was traced, and the internal area was determined in Photoshop CS4. The thickness of the hair cell epithelium was measured as the distance from the lower edge of the BM to the upper edge of the hair cell epithelium at a point directly lateral to the internal spiral sulcus, perpendicular to the BM. The distance between the TM and hair cell epithelium was defined as the shortest distance between the two that is perpendicular to the BM. The thickness of the spiral limbus and OSL was measured from the RM and spiral limbus connection to the lower edge of the OSL, perpendicular to the OSL. The thickness of the RM was measured at the midpoint, perpendicular to the curvature
at that point. Finally, the thickness of the bone and SL was measured at the connection point between the RM and SL, perpendicular to the curvature at that point.

Penetration depth was measured by choosing an A-line that was near the midpoint of where the RM attaches to the spiral limbus. The amount of tissue imaged, as determined by eye, refers to the length of bone and soft tissue minus the length of the fluid-filled space. The image intensity was measured at the apical otic capsule, hair cells, spiral limbus, and perilymph of the ST and was calculated by averaging the pixel intensity within a 10 by 10 pixel box. Weber contrast was calculated by dividing the perilymph intensity (background) from the difference of the either the image intensity of apical otic capsule, hair cells, or spiral limbus (signal) and perilymph. This value was then multiplied by 100 and presented as a percentage. Analysis of variance (ANOVA) followed by two-tailed, non-paired Student’s t-tests were used to assess for statistically significant differences in measurements of distance, thickness, or image intensity between tissues (P < 0.05).

The pixel intensity values across the region of the internal spiral sulcus were recorded from images derived by averaging five consecutive OCT image slices from a single image stack. The intensity values were recorded along a 100 µm line drawn perpendicular to the BM, across the internal spiral sulcus. Two-tailed, non-paired Student’s t-tests were used to determine significance between the pixel intensity values from 0 to 10 µm, 45 to 55 µm, and 90 to 100 µm.

3. Results

3.1 OCT image of an unopened murine cochlea

Using our spectral domain OCT system, we first imaged an excised P15 mouse cochlea. A sample X-Z slice and A-line are shown in Fig. 4A and 4B, respectively. In the OCT image, cochlear structures such as the organ of Corti and stria vascularis can be identified based on our knowledge of cochlear anatomy. These structures can also be identified on the A-line based on their relative depth.

![Fig. 4. (A) OCT image from a P15 mouse cochlea. The bone and soft tissue structures scatter light and produce a signal that is visible with OCT. In contrast, the surrounding fluid does not produce a visible signal. (B) Magnitude and depth plot of the A-line highlighted in yellow in (A).](image)

3.2 The opened adult murine cochlea

We then imaged normal adult mouse cochleae with the apical otic capsule bone removed to minimize unwanted scattering. A representative OCT image, along with a representative paraffin-embedded histological image of an equivalent region of the cochlea, is shown in Fig. 5. In the OCT image, the RM, BM, TM, and modiolus could be clearly identified. Because the apical otic capsule had been removed, the lateral edge of RM was unattached, opening the
The curvature of the TM was away from the hair cell epithelium. The crevice under the attachment of the TM, the internal spiral sulcus, and the space between the IHCs and OHCs, the tunnel of Corti, were also visible. In the histological image, these structures can also be seen, but are often distorted as a result of the fixation and dehydration process. This was extremely apparent in the case of the TM, which appeared to be thinned dramatically. This is a common problem with cochlear histology and occurs because the TM is composed of ~97% water [27]. As evident in Fig. 5, OCT imaging can resolve the soft tissues within the apical turn of the excised, murine cochlea. It also appears to provide a more representative characterization of the in vivo anatomy of the TM than fixed histological sections.

We then imaged cochleae from the three genotypes of transgenic TectaC1509G mice [19] to determine whether OCT can visualize soft tissue changes in the TM anatomy that cause hearing loss. Tecta+/+ mice have a normal TM which attaches to all three rows of OHCs. TectaC1509G/+ mice have a TM which attaches to only the first row of OHCs and suffer moderate hearing loss. TectaC1509G/C1509G mice have a TM that does not attach to any OHCs and suffer profound hearing loss. To ensure more natural cochlear anatomy, we kept the spiral ligament and Reissner's membrane connection intact during the dissection, ensuring that the scala media was not opened. Figure 6 shows representative OCT images of the cochlea from (Fig. 6A) Tecta+/+, (Fig. 6B) TectaC1509G/+, and (Fig. 6C) TectaC1509G/C1509G. We found that the distance between the TM and hair cell epithelium increased with the severity of the mutation, from an average of 16.31 ± 0.63 µm in Tecta+/+ to 23.45 ± 0.76 µm in TectaC1509G/+ and 54.53 ± 4.46 µm in TectaC1509G/C1509G (P < 0.05; Table 1).

To determine if differences exist between OCT and histological images, we made measurements (mean ± SEM) of (1) the area of the TM, (2) the thickness of the hair cell epithelium, (3) the distance between the TM and hair cell epithelium, (4) the thickness of the spiral limbus and OSL, (5) the thickness of the RM, and (6) the thickness of the bone and SL at its junction with RM. Table 1 summarizes the data. Measurements were made on six different images from two different cochleae, except in the OCT case of Tecta+/+ which were made from three different cochleae.
Fig. 6. OCT (left) and paraffin-embedded histological sections (right) of cochleae from (A) Tecta+/+ (Media 1), (B) Tecta+/-C1509G (Media 2), and (C) TectaC1509GCT509G (Media 3). The videos show the image stacks which have been cropped but remain unadjusted. The OCT image of the Tecta+/+ gives the locations of where we made the measurements for (1) the area of the tectorial membrane (TM), (2) thickness of the hair cell epithelium, (3) the distance between the TM and hair cell epithelium, (4) the thickness of the spiral limbus and OSL, (5) the thickness of the RM, and (6) the thickness of the bone and SL at its junction with Reissner's membrane (RM). Since the bone was opened in the OCT images, the actual measurement of the bone and SL thickness was made at another slice of the image stack. Depicted is the approximation of that thickness in the current slice.
Table 1. Measurements of the Soft Tissue Structures Within the Cochlea.

|                      | Tecta<sup>+/+</sup> | Tecta<sup>+/C1509G</sup> | Tecta<sup>C1509G/C1509G</sup> | A       | B       | C       |
|----------------------|---------------------|---------------------------|-----------------------------|---------|---------|---------|
| TM area (µm²): OCT   | 3862.5 ± 144.7      | 4272.92 ± 72.9            | 5443.75 ± 139.9             | ¥ ¥ ¥   |         |         |
| Histology            | 1094.31 ± 322       | 3461.183 ± 399            | 3461.29 ± 340.9             | ¥ ¥ ¥   |         |         |
| Hair cell epithelium thickness (µm): OCT | 115.14 ± 3.73    | 142.94 ± 0.99             | 139.16 ± 3.44               | ¥ ¥ ¥   |         |         |
| Histology            | 44.37 ± 2.31        | 53.30 ± 1.69              | 57.37 ± 4.39                | ¥ ¥ ¥   |         |         |
| Distance between TM and hair cell epithelium (µm): |
| OCT                  | 16.31 ± 0.63        | 23.45 ± 0.76              | 54.53 ± 4.46                | ¥ ¥ ¥   |         |         |
| Histology            | 17.28 ± 4.93        | 10.05 ± 2.33              | 24.24 ± 7.94                |         |         |         |
| Spiral limbus and OSL thickness (µm): OCT | 204.57 ± 6.36      | 218.62 ± 5.87             | 224.36 ± 9.77               |         |         |         |
| Histology            | 151.22 ± 9.69       | 174.13 ± 9.08             | 161.26 ± 11.86              |         |         |         |
| RM thickness (µm):   |
| OCT                  | 20.03 ± 0.88        | 22.66 ± 1.84              | 22.28 ± 0.33                |         |         |         |
| Histology            | 6.21 ± 1.02         | 6.32 ± 0.47               | 7.42 ± 0.88                 |         |         |         |
| Bone and SL thickness (µm): OCT | 105.33 ± 6.45  | 109.4 ± 6.24              | 106.24 ± 6.32               |         |         |         |
| Histology            | 112.88 ± 14.33      | 102.9 ± 7.66              | 109.67 ± 9.77               |         |         |         |

All values are mean ± SEM. Measurements from OCT and histological images are as labeled. * denotes statistical significance between the measurement from OCT and histology. ¥ denotes statistical significance among genotypes, where A is between Tecta<sup>+/+</sup> and Tecta<sup>+/C1509G</sup>, B is between Tecta<sup>+/+</sup> and Tecta<sup>C1509G/C1509G</sup>, and C is between Tecta<sup>+/C1509G</sup> and Tecta<sup>C1509G/C1509G</sup>.

When comparing the measurements made on the OCT images between genotypes, there were also differences between the TM area and hair cell epithelium thickness. For the TM area, the measurement increased with the severity of the mutation. For the hair cell epithelium thickness, the Tecta<sup>+/+</sup> was less than the Tecta<sup>+/C1509G</sup> and Tecta<sup>C1509G/C1509G</sup>; however, the Tecta<sup>+/C1509G</sup> and Tecta<sup>C1509G/C1509G</sup> were not different from each other. The differences in the TM area and hair cell epithelium thickness were reflected in the measurements from the histological images as well, except for between Tecta<sup>+/C1509G</sup> and Tecta<sup>C1509G/C1509G</sup>. Importantly, there were no differences in the spiral limbus and OSL thickness, RM thickness, and bone and SL thickness when comparing between genotypes in both OCT imaging and histology. These were not expected to change. Thus, we conclude that OCT imaging can distinguish between TM differences in mice that contain a mutation responsible for hearing loss in humans.

3.3 Unopened cochlea during development and in adulthood

The mouse cochlea is only partially formed at birth. At P3, the TM is still attached to the hair cell epithelium along its entire width, because the internal spiral sulcus and tunnel of Corti have not formed yet [19]. The otic capsule surrounding the cochlea has not yet undergone endochondral ossification and remains cartilaginous. As such, it should scatter light less than in adult mice. By P15, the organ of Corti is fully mature, and the otic capsule has partially ossified. The adult mouse cochlea (>P30) has a more ossified otic capsule. Therefore, we studied cochleae from P3, P15, and adult mice to assess the abilities of our system to visualize developmental changes in soft tissue morphology and to understand the impact of otic capsule ossification on image quality. Unaltered, representative images are shown in Fig. 7 of a (Fig. 7A) P3, (Fig. 7B) P15, and (Fig. 7C) adult cochlea. In all of the image stacks, the RM, BM, and the modiolus were visible in at least 80% of the apical cochlear turn. Looking at an A-line that was near the attachment of RM to the spiral limbus (Fig. 7A, in yellow), the total depths of tissue imaged in the P3, P15, and adult cochleae were 445.71 ± 16.53 µm (n = 7), 405.63 ±
14.28 µm (n = 8), and 482.5 ± 30.02 µm (n = 6, mean ± SEM), respectively. There was no statistical significance between these measurements. This is summarized in Fig. 7D.

![Image](image.png)

**Fig. 7.** Unaltered, cross-sectional spectral domain OCT images of the cochlea from (A) P3, (B) P15, and (C) adult mice. In all cases, the Reissner's membrane (RM), basilar membrane (BM), and modiolus are visible. An example of the vertical line chosen for measuring the amount of tissue imaged is shown in yellow. Examples of the 10 by 10 pixel boxes used to calculate the signal intensity in a given region are also in yellow. Graphs showing (D) the amount of tissue imaged, (E) the average signal intensity in different regions, and (F) the contrast percentage of regions 1, 2, and 3. Number of samples is noted in (D), and statistical significance is noted in each of the graphs by a paired * or ¥.

The image quality of the soft tissues was reduced in the P15 and adult mouse. This is illustrated in Fig. 7E. The graph depicts the average signal intensity of 10 by 10 pixel boxes. The location where the signal intensity was measured is shown roughly by the numbers 1, 2, 3, and 4 in (Fig. 7A-C). The regions encompass a portion of the apical otic capsule, the hair cells and supporting cell region, the spiral limbus, and the ST, respectively. As would be expected, there was a statistically significant increase in the overall signal in the apical otic capsule with age and no difference in the signal from the ST. Importantly, there was a decrease in the signal from the hair cells at P15 and adult; this is more clearly seen when looking at the contrast in Fig. 7F. There were, however, no statistically significant differences in the signal intensity from the spiral limbus between the different age groups. In the cochlea of the P3 mouse, since the bone has not fully calcified, the inner cochlear structures are better defined.
Additionally, the TM does not lift from the BM in the mouse until after P3 [19]; this is shown in the P3 OCT image by a lack of the internal spiral sulcus. The pixel intensity values across the region of the internal spiral sulcus are graphed in Fig. 8. There was a decrease in the signal around the 50 µm position, representing the fluid filled internal spiral sulcus, in the P15 and adult. The higher signal at the 0 µm position is the organ of Corti and osseous spiral lamina; the higher signal at the 100 µm position is the spiral limbus and TM. Therefore, OCT provides the ability to observe a critical, yet subtle change in the normal development of cochlear anatomy.

Fig. 8. Average intensity OCT images from five slices from the cochlea of the P3 (n = 7), P15 (n = 8), and adult mouse (n = 6). The pixel intensity across the internal spiral sulcus, depicted by the yellow line (100 µm), is graphed. The zero position is closer to the basilar membrane (BM). Statistical significance is noted in each of the graphs by a paired * or ¥.

4. Discussion

We found that spectral domain OCT can provide high-resolution images of the soft-tissue structures critical to normal hearing. Using freshly-excised mouse cochleae, we could perform
routine visualization and assessment of several critical structures, including Reissner's membrane, the basilar membrane, the hair cell region, the tectorial membrane, the spiral ligament, the spiral limbus, and the modiolus. Of greater interest is the ability of OCT to identify anatomic malformations that define the pathophysiology of hearing loss in a mouse model of human disease. In these experiments, OCT was used to image the cochlea at discrete time points throughout the development of the mouse cochlea. Monitoring the morphology of the cochlear soft tissue during the developmental timeline is important not only for our understanding of inner ear maturation, but also for understanding how problems in maturation can lead to congenital malformations. One concern about using OCT to study the inner ear is the impact of the surrounding bone, which is highly scattering. However, our study has shown that while otic capsule ossification affects image quality to a degree, it does not substantially impact the ability to study the internal soft tissue structures in adult mice.

Furthermore, OCT overcomes many of the problems associated with the substantial histological artifact that occurs with fixation, decalcification, dehydration, and embedding of the cochlea. In general, the measurements made from tissue processed by fixed histology were less than from fresh tissue imaged by OCT. We attribute the majority of the differences between the measurements of the soft tissues in the OCT images and in the histological images to dehydration-induced shrinkage. Indeed, a previous study in gerbils has shown that the TM cross-sectional area, as well as that of other cochlear tissues, can shrink dramatically depending on the dehydration protocol [28]. We should note, however, that in the opened cochlea, the TM is no longer in its native environment and its shape can change depending on the ionic imaging solution that is used [29]. Consistent with this notion, structures that have lower water content had similar measurements between the two imaging modalities. In particular, the thickness of the bone and SL measurements in all three genotypes were not different. Imaging time is another benefit of OCT imaging compared to traditional histological sectioning. An entire cochlea can be imaged using OCT within a couple of minutes, whereas the fixation, decalcification, paraffin embedding, sectioning, and imaging associated with histology would typically take a week or more to accomplish.

Most importantly, analysis of the OCT images provided important findings that could not be made by an analysis of only the histological images. When comparing the genotypes, measurements from the OCT images showed statistically significant differences in the distance between the TM and hair cell epithelium. This was not evident from the analysis of the histological images. Furthermore, in our original description of the Tecta mutant mouse [19], we decided against measuring the thickness of the hair cell epithelium in the histological images because we thought those measurements would be tainted by artifact. Our current measurements from both the OCT and histological images suggest that there is indeed an increase in the thickness of the hair cell epithelium in the Tecta<sup>C1509G</sup> and Tecta<sup>C1509G/C1509G</sup> mice. This may reflect the fact that Tecta<sup>C1509G</sup> and Tecta<sup>C1509G/C1509G</sup> mice have an upregulation of the prestin protein within their OHCs [19]. Prestin is a motor protein that produces force to amplify the sound pressure waves within the cochlea [30], and indeed in these mutants, increased prestin results in increased vibratory amplitudes of the organ of Corti [22]. Alternatively, the structure of the hair cell epithelium may have developed differently because of the altered biophysical properties of the overlying malformed TM [21].

Our OCT images of the mouse cochlea at different ages, which were taken without removing or thinning the cochlear otic capsule bone, revealed the expected compositional and structural changes associated with development. These include the endochondral ossification of the otic capsule and the resorption of the inner sulcus cells, freeing up the middle region of the TM. The latter is a key developmental milestone in achieving a functional cochlea.

Our images also compare favorably with previously collected OCT images of the cochlea [31–38]. While the use of longer wavelengths improves the signal-to-noise ratio of deeper structures, it is associated with a loss of axial resolution [36], given a similar bandwidth. Our
approach still allows us to quantitatively measure soft tissue structures and to compare the differences in cochlear anatomy relevant to hearing loss with histological sections.

Future work is needed to assess whether OCT can detect cochlear soft tissue changes in vivo. We believe that at least one half of a cochlear turn in the mouse will be visible given proper surgical access. However, transitioning the technology to use in humans would benefit from coupling the imaging system to an endoscope. Since the otic capsule bone is thicker in humans, visualization through the round window membrane will likely be necessary. Given the success of OCT imaging in other clinical fields, we believe it holds a similar promise as a new tool for assessing the pathophysiology of hearing loss.

5. Conclusion

Spectral domain OCT can detect subtle anatomical changes in the organ of Corti during the course of post-natal cochlear development. As well, OCT produces images of sufficient resolution to allow quantitative measurements of morphology in intra-cochlear soft tissues relevant to hearing in Tecta transgenic mice. Moreover, OCT can provide relevant structural information that is difficult to obtain with conventional histology.

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