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Research Paper

Effect of conservation method on ear mechanics for the same specimen

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ABSTRACT

Background and aims: As an alternative to fresh temporal bones, Thiel conserved specimens can be used in the study of ear mechanics. Conserved temporal bones do not decay, permit long-term experiments and overcome problems with limited access to fresh (frozen) temporal bones. Air conduction motion of the tympanic membrane (TM), stapes (ST) and round window (RW) in Thiel specimens is similar to that of fresh specimens according to reports in the literature. Our study compares this motion directly before and after conservation for the same specimens.

Methods: The magnitude of motion of TM, ST and RW elicited by acoustic stimulation via the external auditory canal was measured using single point laser Doppler vibrometry (LDV) accessed through a posterior tympanotomy. For the initial measurements (10 ears), fresh frozen whole heads were thawed for at least 24 h. Afterwards, the entire whole heads were embalmed according to the Thiel embalming method and measurements were repeated 3 and 12 months later.

Results: The magnitudes of TM, ST and RW motion before and after Thiel conservation differed maximally 10 dB on average. A significant increase in TM motion was observed at low frequencies only after long term conservation (12 months). ST motions decreased significantly between 161 and 5300 Hz after 3 months of Thiel conservation. Over the same time period RW motions decreased significantly between 100 and 161 Hz and 489–788 Hz.

The ST and RW motions across all measured frequencies were lower after 3 months by 5.7 dB and 7.1 dB, respectively, without further changes after 12 months of conservation. The mean phase shift between ST and RW motion was only 2.1° for frequencies below 450 Hz.

Discussion and conclusion: Thiel embalming changes motion of TM after long term conservation. ST and RW motion changed mainly after short term conservation. The phase shifts close to 180° between ST and RW motion indicates that the cochlea was still filled with liquid without air bubbles. The results show that Thiel conserved specimens can be used as an alternative model to fresh frozen preparations with some limitations when studying mechanics of the normal human ear, for example, in implant design.

1. Introduction

Micromechanics of the human ear are mainly studied in human temporal bone specimens (Goode et al., 1993; Rosowski et al., 1990; Voss et al., 2000). The motion of the tympanic membrane can be measured in humans without anesthesia while other ear structures are only accessible during ear surgery (Chien et al., 2006; Huber et al., 2001).

Abbreviations: TM, Tympanic membrane; ST, Stapes; RW, Round window; LDV, Laser Doppler vibrometry; mm/s/Pa, Millimeter/second/Pascal; ASTM, American standard practice for describing system output in implantable middle ear hearing devices; CI, Confidence interval; STD, Standard deviation.

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While fresh frozen human specimens are often used as the first choice for studying ear motions, the duration of experiments is limited due to biological degradation. This would not occur for conserved specimens e.g. using formalin. Additionally, in some institutions the supply of fresh or fresh frozen human temporal bone specimens is limited because of regulatory restrictions or ethical concerns. For these reasons, over 10 years ago, we started using anatomical specimens embalmed in Thiel solution as tissue properties remain closer to those of live tissue compared to formalin conservation (Burkhart et al., 2010; Quester and Schroder, 1997; Wilke et al., 2011). First, Thiel conserved specimens were used in the development of an implantable hearing device (Hausler et al., 2008). Subsequently, the tissue properties were found to have characteristics permitting laser Doppler velocity (LDV) measurements of the tympanic membrane (TM), stapes (ST) and round window (RW). Measurements were similar to those in live subjects and fresh temporal bones (Stieger et al., 2012). Thus using conserved specimens would facilitate the planning of experiments. To establish reproducibility of setups enables long-duration protocols (Stieger et al., 2012) and lengthy studies of different parameters essential for the development of hearing implants (Arnold et al., 2010; Dobrev et al., 2019).

While it was shown that using conserved specimens has some advantages concerning measurement durations, earlier studies did not compare the influence of Thiel conservation in the same specimen. In the present study we provide a direct comparison before and after conservation based on LDV measurements of the TM, ST and RW. We used the ASTM standard to check the integrity of temporal bones for LDV measurements at the level of the ST. The ASTM standard (ASTM, 2005) provides an absolute value of the ST magnitude when acoustic sound is applied to the external auditory canal. Furthermore, the two-window hypothesis of a fluid filled cochlea assumes that the volume velocity of the ST equals the volume velocity of RW. This implies that a mechanically intact cochlea shows a phase difference of 180° (oppositely directed) between ST and RW (Stieger et al., 2018). Therefore, the phase difference between RW and ST motion was used as another quality control.

2. Material and methods

2.1. Temporal bone specimens

The study was performed on 12 normal ears of six fresh frozen human whole head specimens (Science Care, Phoenix (AZ), USA; mean age 66 years, range 34–75 years, three male, three female). Initially, a microscopic inspection of the external auditory canal and the tympanic membrane was performed and any debris was removed using suction. The use of the whole head specimens was approved by the regional ethics commission (EKNZ BASEC 2016–00,599).

2.2. Surgical preparation of specimens

The preparation of the specimens followed the protocol described by Stieger et al. (2012). In summary, while preserving the pinna and the external auditory canal, we performed a conventional mastoidectomy and facial recess approach. The mastoid segment of the facial nerve was removed for optimal access to the RW niche for LDV measurements. The stapedial tendon was dissected to allow targeting the laser on the posterior crus. In some ears, adhesions limiting the view to the RW were carefully removed. In two ears the RW membrane was perforated during the preparation resulting in 10 ears (six right, four left) being used for the measurements.

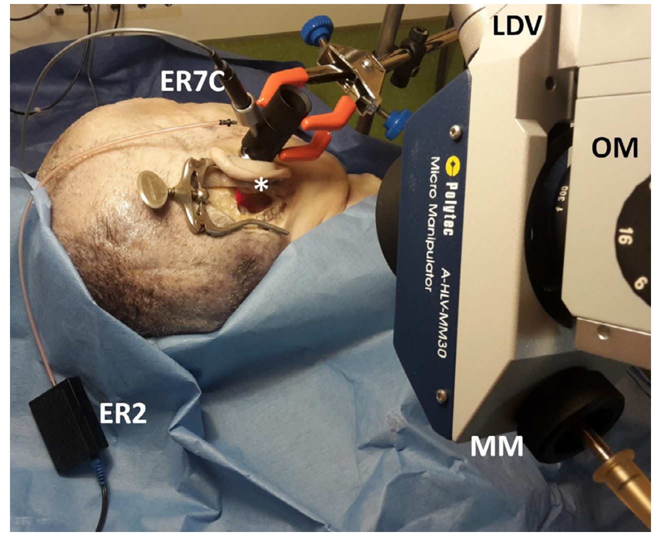

Fig. 1. Measurement setup. The glass-backed coupler (black cylinder) placed in the external auditory canal comprises the insert earphone ER2 and the microphone ER7C. The laser Doppler vibrometer (LDV) is coupled to the operating microscope (OM) and the laser beam is positioned with the micro manipulator (MM). Asterisk (*): marks the most lateral point of the posterior auditory canal wall which limits the maximal aiming angle of the LDV beam to the posterior crus.

2.3. Measurement setup

The specimens were placed on an optical breadboard with vibration absorbing pads (Thorlabs, Newton, NJ, USA). A glass-backed coupler with an insert earphone (ER2; Etymotic Research, Elk Grove Village, IL, USA) and a probe microphone (ER7C, Etymotic, Elk Grove Village, IL, USA) were entered into the external auditory canal and acoustically sealed with plasticine. The LDV (CLV a25–44, Polytec, Waldbronn, Germany) was attached to an operating microscope. A joystick-controlled aiming prism (HLV MM2, Polytec, Waldbronn, Germany) allowed positioning the laser beam on the TM, ST, RW and promontory (Fig. 1). Microbeads were placed at the umbo, posterior crus of the stapes, at minimum three positions on the RW and on the promontory. For the RW one microbead was positioned in the center. The other microbeads were radially positioned between the center and the border of the RW niche in arbitrary angular positions. The positions of the microbeads were documented by sketches to assure reproducibility of the experimental setup after 3 and 12 months.

Consistent angle of the LDV beam is important for reproducible setups. For the TM measurements, the variation of the angle is determined by the borders of the external auditory canal which naturally defines the view onto the umbo.

The ST velocity was measured transmastoidally at the posterior crus after removal of the stapedius tendon as described above. The LDV beam was in plane of the two stapes crurae. The aiming angle of the LDV beam for ST was limited by the most lateral point of the posterior auditory canal, which provides a consistent landmark for comparable stapes measurements in each ear (Figure 1). Thus, the angle was approximately 60° in relation to the footplate in order to acquire predominately piston-like ST motion for all measurements. For all measurement of the RW velocity the LDV beam was perpendicular to the RW. Promontory velocity was measured with the same viewing angle as the RW. We did not compensate for the viewing angle throughout the entire study.

2.4. Stimulus generation and signal acquisition

Thirty logarithmically distributed pure tones in the frequency range of 100 to 10,000 Hz with a density of 4 points per octave were sequentially applied in the external auditory canal with a constant stimulus voltage. Each frequency was repeated 10 times. The internal signal generator of the UPV system (R&S UPV, Rohde & Schwarz, Munich, Germany) was connected in series with an attenuator (Tucker Davis Technology, Alachua, FL, USA) and a custom-made amplifier. The amplifier (attenuator at 0 dB) was initially set to a maximum output stimulation level that was adapted to prevent overloading of the transducers. For all stimulation levels only the attenuator level was changed to either 0 or 10 dB. The electrical signal stimulus of 3.16 and 1 V was therefore delivered to the ER2 transducer. A 10 dB higher stimulation level resulted generally in 10 dB higher motion with a mean deviation of 0.2 dB (range −0.18 to 1.00) for fresh frozen specimens and 0.1 dB (range −1.1 to 1.8) after 3 months of Thiel conservation, indicating that stimulation levels were applied within a linear range (250 to 4500 Hz). For frequencies below 250 Hz and above 4500 Hz the LDV signal was close to noise levels with 1 V (attenuator 10 dB) stimulation. The analysis was based solely on measurements with 3.16 V stimulation.

The sound pressure level (SPL) was measured close to the tympanic membrane with a microphone (ER7C). For 3.16 V stimulation, the SPL ranged between 89 and 106 dB SPL between 100 and 10,000 Hz in all specimens. For the vibration measurements the LDV decoder was set to a sensitivity of 2 mm/s/V, with a highpass-filter at 100 Hz and a lowpass-filter at 20,000 Hz. Transfer functions (mm/s/Pa) were calculated between the acoustical stimulation and the TM, ST, and RW velocity. We measured a delay of 0.97 ms between ER7C and the LDV signal which was compensated for when calculating phase shifts.

2.5. Experimental protocol

Experiments were first conducted with the specimens in fresh frozen condition and repeated after fixation of the specimens according to Thiel (1992).

The fresh frozen whole head specimens were defrosted for 24 h and prepared for LDV measurements. As stated above, measurements of the TM were performed with 1 V and 3.16 V to check linearity. The experiments where continued only when response magnitudes differed by 10 dB. Sound pressure was continuously monitored to detect potential accumulation of embalming fluid in the external auditory canal.

At the beginning of each experiment, phase shift between ST and RW was determined in every ear to exclude the presence of a false round window membrane or a third cochlear window (Stieger et al., 2013).

Then ST and RW measurements were conducted. Additionally, we measured the motion of the promontory, when acoustic stimulation was applied to the TM. The ambient noise level was measured without acoustic stimulation at the TM.

Specimens were placed in a Thiel solution bath (proportional composition: hot water 100 ml, boric acid 3 g, ethylene glycol 10 ml, ammonium nitrate 10 g, potassium nitrate 5 g, 4–chloro-3-methylphenol 2 ml, sodium sulfate 7 g, formalin 2 ml) for at least 3 months to ensure thorough conservation. We removed the specimens from the Thiel bath over night for excess fluid to drain. Inspection of external auditory ear canal including the tympanic membrane was performed as in the fresh frozen condition and residual Thiel solution was aspirated. The LDV measurements were repeated after placing new microbeads on TM, ST and RW at the documented positions.

Afterwards, the specimens were put back into the Thiel bath for another 9 months until long-term measurements were performed using the same protocol.

The influence of conservation according to Thiel on ear mechanics has been studied by our group (Stieger et al., 2012). However, there have been no reports with similar analyses for specimens conserved with pure formalin. Formalin is known to change the mechanical properties of tissues (Burkhart et al., 2010) which may be the reason why research in ear mechanics is usually not performed with formalin conserved specimens. For the sake of completeness, we compared the fresh frozen and Thiel conserved conditions with formalin in a single specimen. After the first measurement with Thiel conservation (i.e. after three months), one head was embalmed in formalin 6% for another three months. Then the LDV measurements of TM, ST and RW motion were repeated. In this head only one ear was available for measurements because the opposite ear became unusable after RW membrane damage during the preparation.

2.6. Data analysis and statistics

UPV raw amplitude and phase data of velocity and sound pressure were imported into Microsoft® Excel® 2016 (Microsoft Corporation, Redmont, WA, USA) for further analysis. The transfer function between velocity and sound pressure for the ST motion was compared to the ASTM standard. For the final analysis of the RW motion we selected for each ear the microbead position which showed the highest overall magnitude in the fresh frozen condition. For descriptive statistics and visualization geometric mean and confidence intervals (CI) for TM, ST, and RW were calculated for all conditions of specimens. Gain ratios for TM, ST and RW are calculated for further statistical analysis. We used non-parametric one sample Wilkoxon-sign-rank statistical tests for all frequencies to check if changes of median values deviate significantly (Prism 8.4.3, GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Transfer functions

Fig. 2 shows the magnitude of the transfer functions of all specimens and population averages for TM, ST and RW for fresh frozen specimens and after these specimens conservation in Thiel solution for three and 12 months. For ST measurements, additionally the ASTM mean and CI are shown. It can be observed that the variation of the RW motion was larger than that of the TM and ST for all conditions.

The ST motion for fresh frozen specimens was within the range of ASTM for most stimulus frequencies. After Thiel conservation the ST magnitudes were at the border of the ASTM standard.

Fig. 3 shows the mean amplitude and CI for all three conservations. The magnitude of the TM gain after 12 months of conservation tended to be higher for frequencies below 800 Hz than for the other conditions but these were still within the CI.

The ST magnitudes were similar in all conservations below 800 Hz. For the frequencies between 800 and 3000 Hz the ST magnitude after Thiel conservation was more than one CI lower compared to the fresh frozen condition. The maximum difference was 9.9 dB. The same effect of lower magnitude after conservation in the mid frequency range (maximally 6.7 dB) was also found in the RW but with a larger CI.

The mean phases of the TM, ST, RW are shown in the lower row of Fig. 3 for all conservations. Generally, the phase decreased approximately 3 cycles (1080°) between 100 and 6000 Hz. The maximum differences of the phase means for the three conservation condition below 6000 Hz were 4°, −21° and 28° for the TM, ST and RW, respectively. The RW is shifted by approximately ½ cycle (180°) when compared to the ST and TM (see also 3.3).

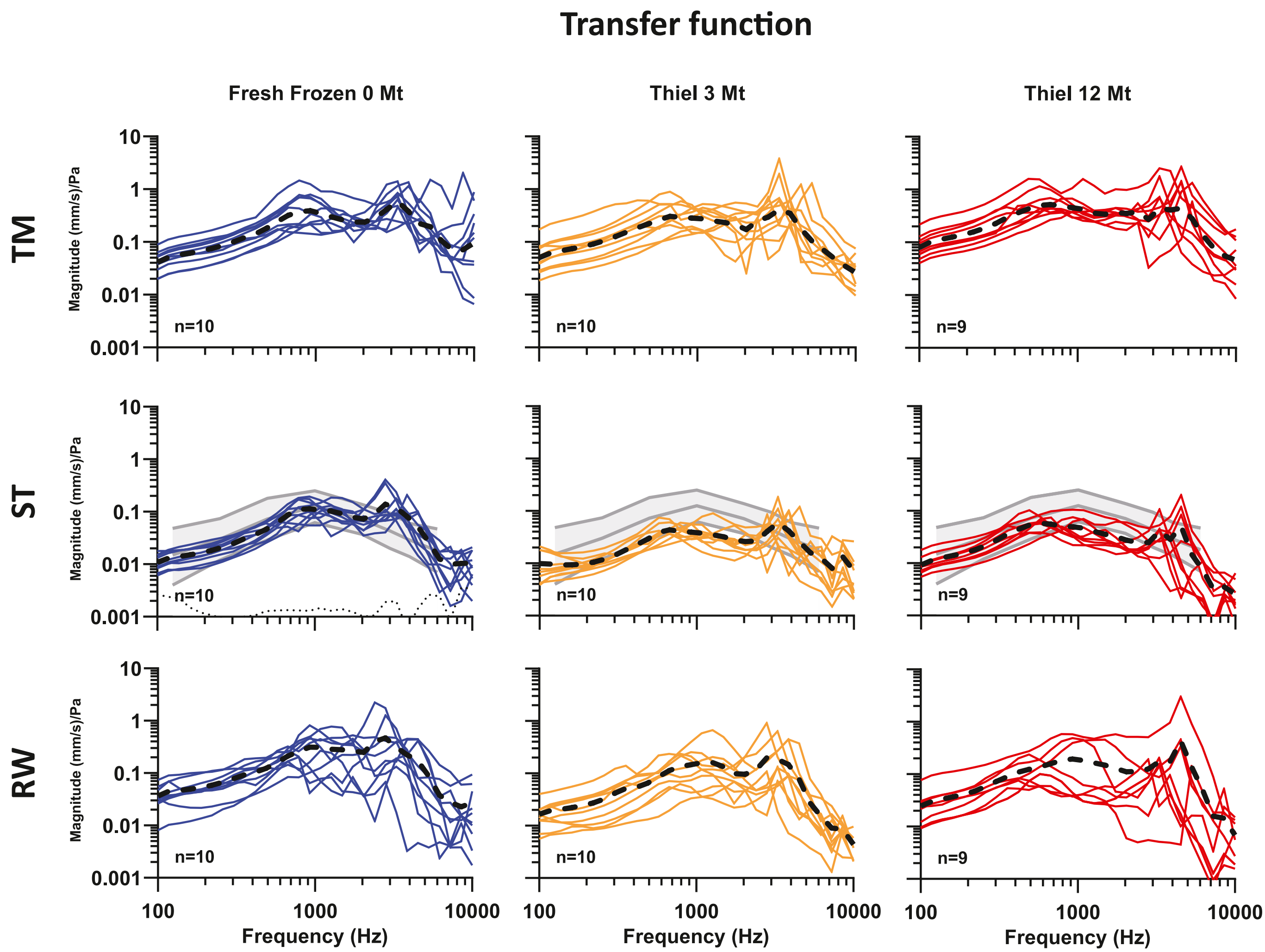

Fig. 2. Transfer function of tympanic membrane (TM) stapes (ST) and round window (RW) in different conservation conditions: thawed fresh frozen (left, blue), after 3 months of Thiel conservation (center, orange), after 12 months of Thiel conservation (right, red). Thin lines: individual specimens. Dashed black line: geometric mean. Gray: range of ASTM-standard for ST velocity. Dotted line in ST fresh frozen 0 Mt represents the mean transfer function on promontory ($n = 8$).

3.2. Ratios of transfer functions

Fig. 4 shows the change in TM, ST and RW motion between all the stages of conservation.

The averaged TM magnitude across frequencies after 3 months of Thiel conservation shows a mean difference of −0.7 dB (STD 2.7, range −9.2 to 2.5) compared to the fresh frozen condition. Two frequencies (5300 and 10,000 Hz) differ statistically significant from zero. After further conservation of 12 months the mean difference was 4.5 dB (STD 1.8, range −0.5 to 8.5). Statistic significant differences were observed between 161 and 574 Hz as well as between 1740 and 2400 Hz. The mean difference between the initial fresh frozen condition and 12 months of Thiel conservation was 3.0 dB (STD 3.7, range −7.4 to 4.6). Statistic significant differences of TM magnitude were observed between 100 and 574 Hz.

The averaged ST magnitude across frequencies after 3 months of Thiel conservation was 5.7 dB (STD 3.0, range −10.1 to 1.7) lower than in the fresh frozen condition. The largest differences were observed between 800 and 3000 Hz with a maximal value of −10.1 dB. Statistically significant differences are observed in the frequency range between 161 Hz to 5300 Hz. The further conservation of 12 months in Thiel solution increased the ST magnitude by 0.4 dB (STD 4.2, range −9.9 to 5.3). Statistically significant differences were observed in the low frequency range between 356 Hz and 672 Hz and for two measurements at high frequencies (7280, 8530 Hz). The mean difference between the initial fresh frozen condition and 12 months of Thiel conservation was −5.4 dB (STD 4.7, range −13.6 to 1.2). Statistic significant differences of ST magnitude were observed between 924 and 10,000 Hz except at the frequencies 4520 and 6210 Hz.

The averaged RW magnitude across frequencies after 3 months of Thiel conservation was 7.1 dB (STD 1.4, range −1 to −10.0) lower than in the fresh frozen condition. Statistically significant differences were observed in two frequency bands (100–161 and 489-788 Hz) and two single frequencies (1740, 10,000 Hz). The further conservation of totally 12 months in Thiel solution increased the RW magnitude with an averaged difference of +1.9 (STD 3.4, range −6.0 dB to 6.7 dB. Statistically significant differences were observed only at one frequency, 418 Hz. The mean difference between the initial fresh frozen condition and 12 months of Thiel conservation was of -5.6 dB (STD 2.9, range -11.9 to -0.9). The only statistic significant difference of RW magnitude was observed at 1080 Hz.

The averaged magnitude curve shapes were similar for the ST and the RW. The variability of individual measurements was larger for the RW than for the ST.

Promontory motion for the maximal stimulation level (3.16 V) barely surpassed the ambient noise level (approximately by 2–

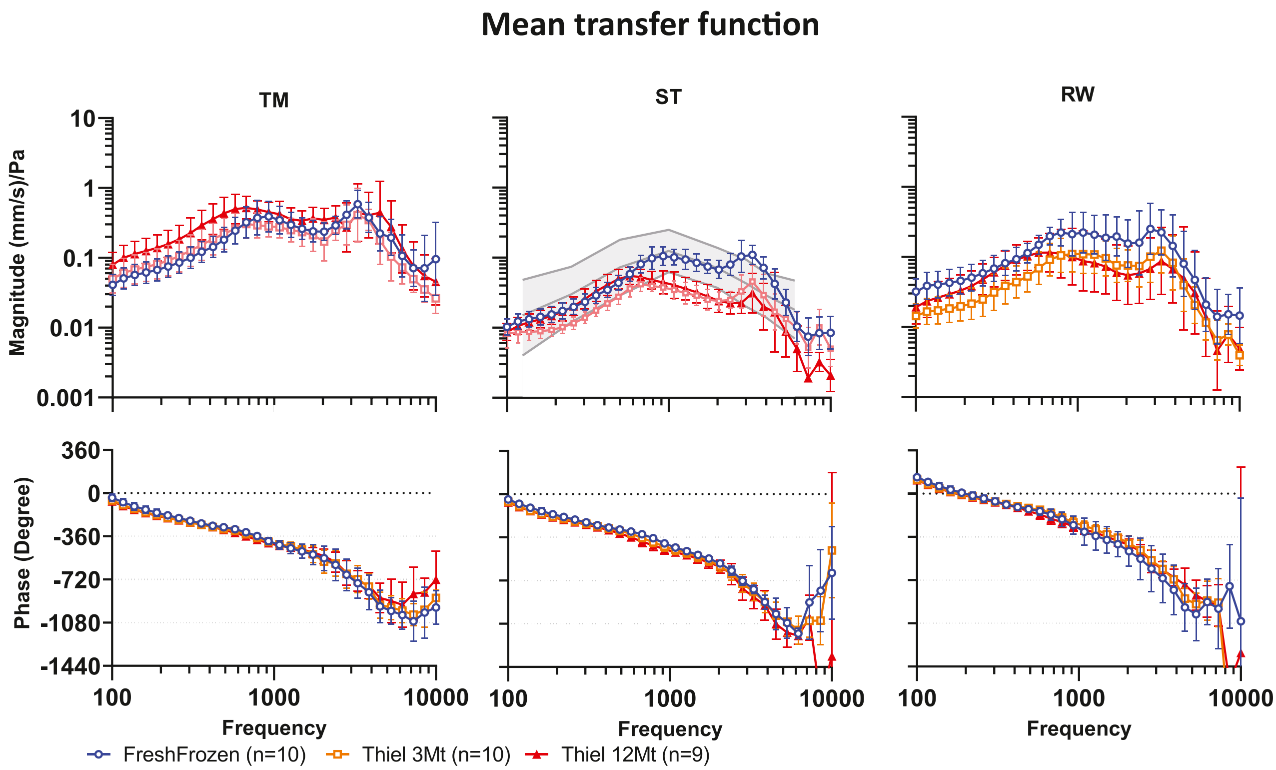

Fig. 3. Mean transfer function magnitude (upper row) and phase (lower row) at tympanic membrane (TM), stapes (ST) and round window (RW). Different conservation conditions: thawed fresh frozen (blue circles), after 3 months of Thiel conservation (orange squares), after 12 months of Thiel conservation (red filled triangles). Vertical-bars define 95% confidence interval. Gray: range of ASTM-standard for stapes velocity.

3 dB). Therefore, we used the transfer function of the promontory to estimate the noise floor level.

The mean difference in promontory motion between fresh frozen and Thiel conserved specimens was 1.2 dB. In the mid frequency range between 450 Hz to 4500 Hz the promontory motion was at least 23 dB lower than the ST motion.

3.3. Phase difference

Fig. 5 shows the phase difference between ST and RW motion for all conditions. We observed oppositely directed motion (180°) between ST and RW at low frequencies. The mean deviation was small in general for low frequencies. In the fresh frozen condition, we found a mean phase deviation from 180° of 2.1° (range −3.5 to 6.1) for frequencies below 450 Hz. After 3 month and 12 month of Thiel conservation the mean phase deviations from 180° below 450 Hz were 0.9° (range −4.8 to 3.2) and 0.7° (range −4.3 to 3.5) respectively. The smallest variation among individual phase measurements was observed after 12 month of Thiel conservation.

3.4. Formalin conservation

Formalin conserved specimens differ from fresh frozen and Thiel specimens in several aspects. The skin remains soft and elastic after Thiel conservation whereas the skin is of leather-like consistency after formalin conservation. Furthermore, there was no visible change to the RW after Thiel conservation whereas the RW became non-transparent after formalin conservation.

Fig. 6 shows the effect of formalin conservation in one ear. TM motions were of the same order of magnitude as Thiel conserved specimens apart from an additional resonance at 1270 Hz. This resonance was also observed in ST and RW after formalin conservation. Frequencies above 1000 Hz showed similar ST motion as after 3 months of Thiel conservation. Below 1000 Hz the ST motion was substantially reduced by at least 20 dB.

In contrast to the ST motion, the RW motion was substantially reduced for all frequencies below 8000 Hz. In fact, RW motion surpassed noise level only for frequencies above 1000 Hz.

4. Discussion

We compared air conduction stimulated motion of the TM, ST and RW in thawed fresh frozen specimens with that of the same specimens which were subsequently embalmed in Thiel solution. Results differed no more than 10 dB on average across preparations and showed the least deviation for TM. Phase measurements showed a 180° shift between ST and RW, indicating a mechanically intact cochlea (Stenfelt et al., 2004).

We improved on our previous study (Stieger et al., 2012), which compared Thiel conserved specimens to data in the literature data on fresh temporal bones and living specimens, by investigating the same heads before and after embalming. Compared to the previous study we observed similar patterns of responses. But the repeated measurements within the same specimens allowed in this study more detailed statistics with matched paired comparisons.

The closest similarity (average −0.7 dB) was found for TM measurements. Nevertheless, a significant increase of the TM motion was observed at low frequencies (161 −574 Hz) after 12 months of Thiel conservation. This could occur for three potential reasons: 1) The diffusion of Thiel solution into the complex structure of the TM might be different to the diffusion of the Thiel solution into other structures such as ligaments and tendons attached to the ossicles. The compliance of the TM might have increased slowly and was therefore only observed after 12 months of Thiel conservation. 2) The microbeads might have been positioned slightly different at the umbo. 3) The compliance of the soft tissue of the external auditory canal might have changed due to the Thiel conservation.

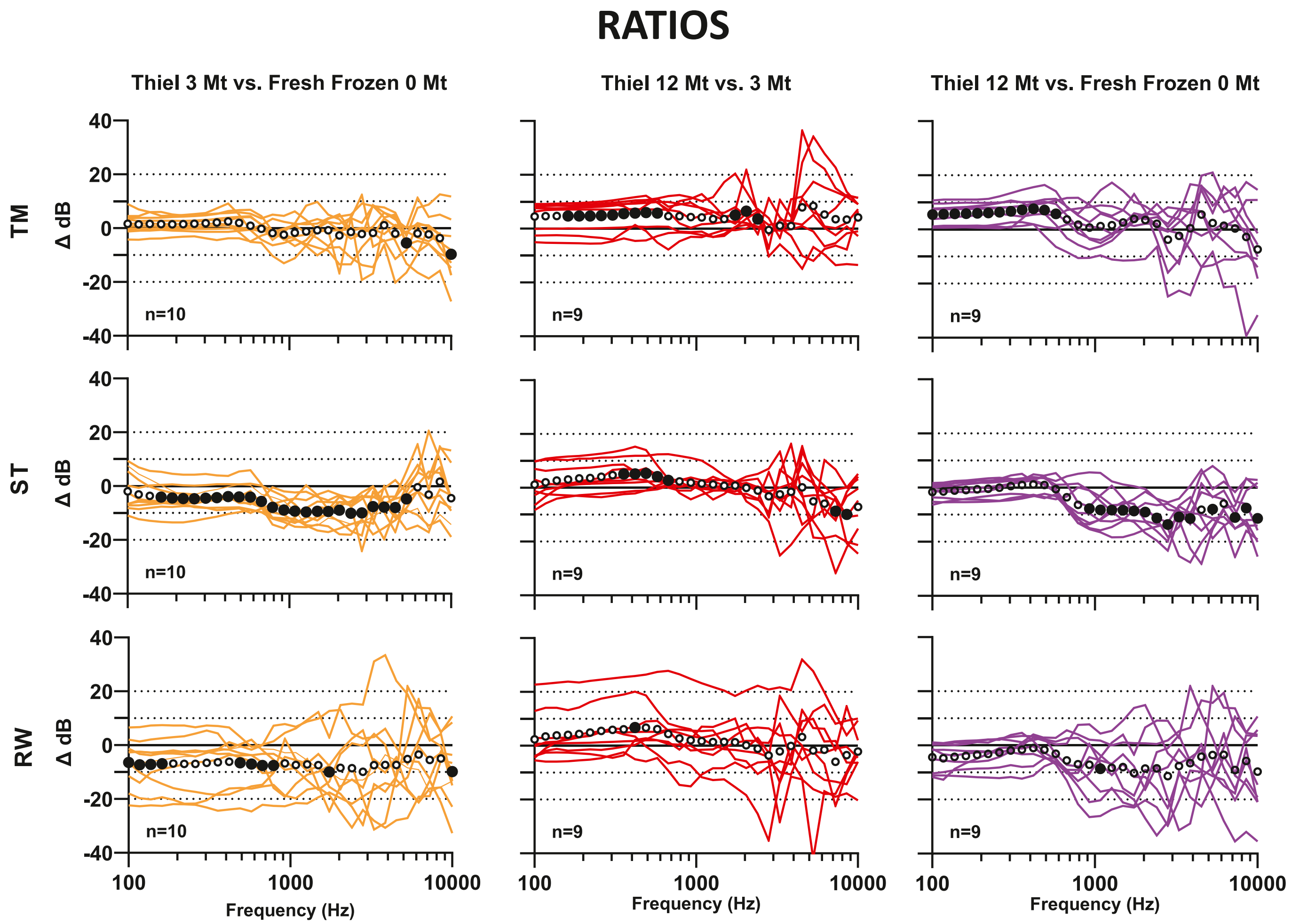

Fig. 4. Ratio of magnitude difference between fresh frozen and Thiel conservation after 3 months (left), between Thiel conservation after 3 and 12 months (middle) and between fresh frozen and Thiel conservation after 12 months. Tympanic membrane (TM, top), stapes (ST, middle), round window (RW, bottom), averaged magnitude:, significant differences: full black bullets, individual specimens: solid lines.

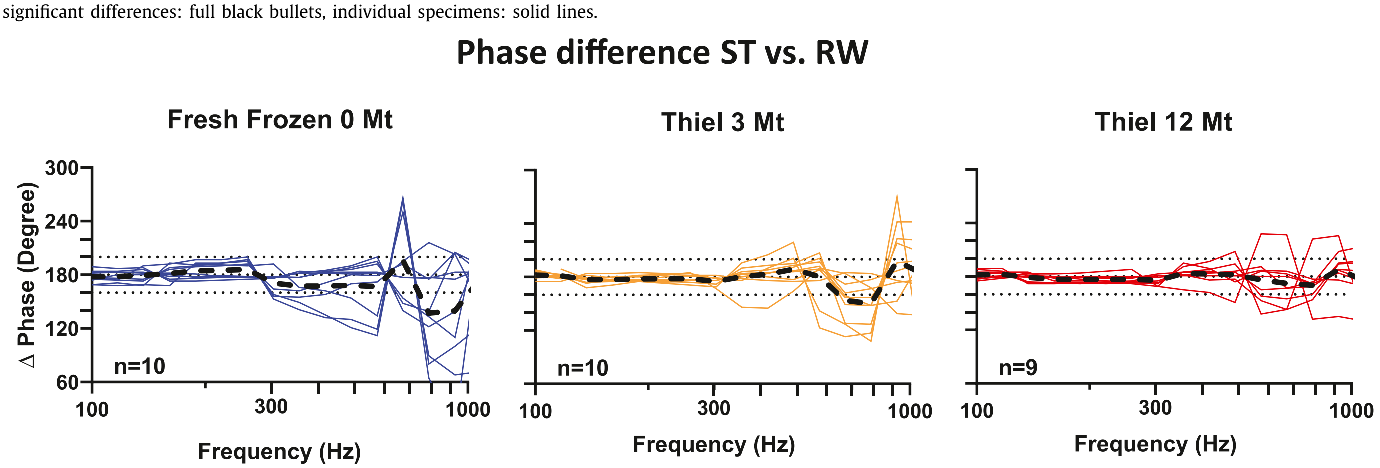

Fig. 5. Phase difference between stapes (ST) and round window (RW) for the frequency range between 100 and 1000Hz: fresh frozen (blue, left), Thiel 3 months (orange, center), Thiel 1 year (red, right). Dashed black line: mean value. Dotted lines: 160° and 200°.

ST and RW motions showed more variability than TM measurements, with a dent in the mid-frequency range as reported by Stieger et al., 2012. However, the mean difference for ST and RW between fresh and Thiel specimen was smaller in the current study (< 10 dB).

In contrast to TM observations, significant changes in ST motion occurred mainly in the first three months. In contrast to the former study (Stieger et al., 2012), a significant decrease in ST motion was observed in a larger frequency range from 161 to 5300 Hz. One reason why the wider frequency range is enhanced is possibly because our statistical analysis using paired comparison within the same specimen. The reason for the averaged decrease of 10 dB might be due to the stiffening of the ligaments and tendons in the middle ear, resulting from the approximately 1.5% of formalin in the Thiel solution.

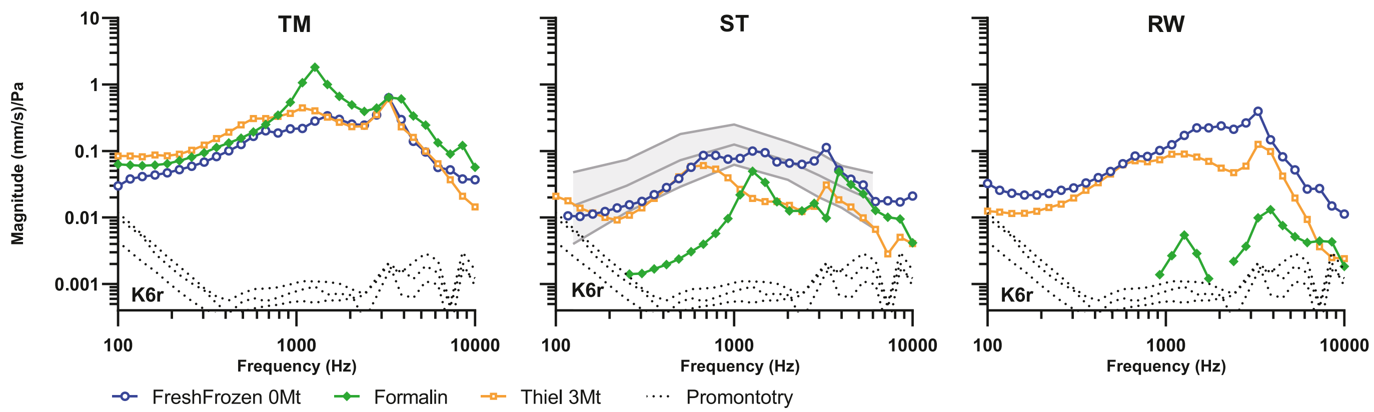

Fig. 6. Transfer function gain on tympanic membrane (TM), stapes (ST), and round window (RW) of one ear, which was first measured fresh frozen (blue circles), then conserved in Thiel for 3 months (orange squares), and then was conserved in Formalin for 3 months (green filled diamonds). Gray: ASTM. Dotted: promontory measurements (fresh frozen, Thiel, Formalin).

Generally, RW measurements showed higher variations which was to be expected because of complex spatial modes present especially at high frequencies. Similar to the ST, significant changes in RW motions were mainly seen in the first three months of Thiel conservation, but with less frequencies at a significant different level mainly due to the higher measurement variation.

Having multiple reflecting beads on the RW exactly at the same position for the experiments on the fresh frozen specimens and after 3 and 12 months of Thiel conservation could potentially reduce measurement variations. Eventhough we documented the position of the reflecting beads at the initial measurement meticulously a different positioning of a fraction of a millimeter for the repeated experiment was inevitable. Comparison of the complex motion would require a grid of beads (Stenfelt et al., 2004) or a scanning Laser Doppler equipment. This might also explain why there is no standard available for RW measurements such as the ASTM for ST measurements.

The final analysis of the current study included 10 RW measurements from 10 experiments whereas only 7 out of 21 RW measurements where valid for comparison in our previous study (Stieger et al., 2012). A possible explanation for better RW responses could be the age of the donors which was 14 years younger on average in the current study compared to the previous study (average age was 66 vs. 80 years). Another reason might be the shorter conservation time (maximum 12 months vs. 48 to 104 months; average 76 months). Therefore, when using Thiel specimens from anatomy for middle ear experiments, it seems advisable to use specimens with shortest possible conservation time.

In our opinion, an important current finding is the long-term stable phase shift of 180° between ST and RW. An earlier study of third window effects reported a change of the RW phase of approximately 0.1 periods i.e. 36° for frequencies below 500 Hz while ST phase remained stable (Chien et al., 2007). In the present study the mean phase difference was below 20°. However, for the individual measurements in the fresh frozen conditions phase differences larger than 20° could be observed. After Thiel conservation the individual variation for all phase differences between ST and RW remained smaller than 20° for frequencies below 300 Hz. The variation is further reduced after 12 month of Thiel conservation. A possible explanation for the reduced individual variations after longer Thiel conservation might be diffusion of Thiel solution into the cochlea. This observation indicates that the cochlea was still filled with liquid without air bubbles or third windows leakage after the conservation, which is a prerequisite when using conserved specimens for studies of ear mechanics (Frear et al., 2018). This supports the notion that Thiel conserved specimens might be used also for RW stimulation experiments (Arnold et al., 2010) A 180° phase shift between ST and RW is also a prerequisite for intracochlear pressure measurements (Nakajima et al., 2009; Stieger et al., 2013). However, further investigation would be needed to show the practical use of Thiel conservation in intracochlear pressure measurements. As reproducible results over a period of nine months have been demonstrated in the current study, enlarged parametric studies with different couplers or devices in the same specimens are feasible (Dobrev et al., 2019).

In our experiments, we conserved the specimens in Thiel solution after transmastoidal posterior tympanotomy without intravascular infiltration of Thiel solution. However, we did not observe major differences in magnitude of TM, ST and RW motion compared to ears from bodies previously conserved according to the standard procedure with intravascular infiltration (Stieger et al., 2012; Thiel, 1992).

For acoustic measurements we constantly observed a magnitude peak around 2–5 kHz with the result that fresh frozen measurements showed higher magnitudes than the ASTM standard. In our setup, the tip of the probe microphone ended in the speculum (7.2 mm outer diameter) in the glass-backed coupler resulting in a larger distance to the TM (4 - 7 mm) with more variation than in the ASTM standard (2 - 3 mm) (ASTM, 2005). Although our magnitude was higher than the ASTM standard, the result of the comparison between the conditions were valid as we used always the same setup.

As expected, the ST and RW measurement were substantially reduced after conservation with formalin over a large frequency range. In contrast TM motion seems to be unaffected. RW was just above noise for frequencies higher than 1000 Hz. However, ST motion of formalin conservation reached the magnitude levels before and after Thiel conservation for frequencies higher than 4000 Hz and 1000 Hz respectively. Although we performed this comparison only in one specimen the aforementioned results indicate that formalin conserved specimens might be used for TM measurements over a wide frequency range and for ST motion measurement at high frequencies (Sutor et al., 2012).

5. Conclusion

Thiel conservation changes the motion characteristics of the ST, RW and TM transfer functions while the windows of the cochlea remain mechanically intact. Considering these characteristics Thiel conserved ears can be used e.g. for comparative parametric studies in the design processes of hearing implants.

Declaration of Competing Interest

None.

CRediT authorship contribution statement

Lukas Graf: Data curation, Formal analysis, Visualization, Writing - original draft. **Andreas Arnold:** Formal analysis, Methodology, Writing - review & editing. **Kourosh Roushan:** Conceptualization, Data curation. **Flurin Honegger:** Formal analysis, Writing - review & editing. **Magdalena Müller-Gerbl:** Conceptualization, Methodology. **Christof Stieger:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Writing - original draft, Writing - review & editing.

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