TORP with fat around the footplate (termed "Pros_FPF"): the fat harvested from the temporalis fat pad was used to surround the prosthesis shaft on the stapes footplate. In all cases the distal bulged end of the TORP was completely covered by fat resulting in complete coverage of the oval window in all cases. This condition was chosen to predict the amount of dampening of sound one might expect when using fat to stabilize the distal end of the TORP on the footplate.

TORP with fat filling the middle ear (termed "Pros_-MEF"): the conditions for "Normal_MEF" were repeated with the TORP in place.

Laser Doppler vibrometer setup and recording software

A LDV (PSV-400 scanning head, OFV-5000 controller, Polytec PI, Tustin, CA, USA) and software (Polytec Scanning Vibrometer version 8.7) were used to measure and record movement of the RWM.

Settings of the software system:

- Average of 4 trials per point
- 640 ms recordings window with a sample frequency of 25.6 kHz
- Acoustic stimulation used was constant sine waves at the following frequencies: 250, 500, 1000, 2000, 3000, 4000, 6000, 8000 Hz
- Sound was presented in the EAC using the ER-3A speaker at 100 dB SPL (or to a maximum of 4 V input if 100 dB could not be attained). This intensity was confirmed with the ER-7C microphone.

Physical setup for LDV measurements: (Fig. 1c)

- The bone was placed in a soundproof booth.
- The temporal bone specimen and scanning head were supported by a stability table to dampen floor vibrations.
- Modeling clay was used to seal the lateral EAC and the holes around the ER-3A and ER-7C to provide an air-tight seal in order to attain the desired sound pressure levels within the EAC. Air-tight closure was confirmed when sound pressure emitted by the ER-3 into the EAC was measured to be at 100 dB SPL by the ER-7.
- The bone was oriented to have a direct full-on view on the RWM in order to most accurately measure its vibration velocity with the LDV.
- 5–7 points were arbitrarily placed over the entire area of reflective tape on the RWM.
- Where visibly possible (all conditions other than "fat filling ME" condition) 2–3 points were also chosen along the stapes or prosthesis, depending on the condition.
  - Setup did not provide the optimal angle for this measurement in every case but, when possible, measurement was completed for phase comparison with RWM movement. In all comparisons, the stapes and RWM were about 180° out of phase at low frequencies.
- The vibration velocity of the RWM and TORP/stapes, when possible, were measured by the laser in response to acoustic stimulation delivered through the EAC.

Data analysis

RWM vibration velocities were normalized to sound pressure (m/s/Pa), converted to dB re:1 μm/s/Pa and analyzed using SPSS 23 software (IBM, Armonk, USA). A two-way repeated measures analysis of variance (ANOVA) was performed with Greenhouse-Geisser corrections for three different condition groupings: 1) Normal-NF vs Normal_MEF; 2) Normal_NF vs Pros_NF; and 3) Pros_NF vs pros_FPF vs Pros_MEF. The within-subject factors that were considered were condition and frequency, with post hoc contrasts for condition being simple type (reference of Normal_NF for tests 1 and 2, Pros_NF for test 3). The middle ear has a known frequency response curve. Therefore, any significant frequency main effects are not important results. However, significant condition × frequency interactions are more relevant effects, indicating frequency-specific differences between conditions. The three different ANOVAs were performed rather than just one containing all conditions in order to better interpret any potential frequency interaction effects and because different reference conditions were desired for examining different contrasts. This approach allowed us to only examine effects which were most relevant post hoc, avoiding having to perform 40 (5 conditions × 8 frequencies) comparisons, which would have increased the probability of a type I error. Type I error rate for the three ANOVAs was reduced to 0.05/3 = 0.0167.

Results

Fourteen temporal bones were dissected. Because of technical issues during dissection (ex: TM perforation, accidental footplate removal with stapes suprastructure excision), some bones were unable to be used for certain conditions. Figure 2 details which bones had data available for each condition.

Normal_NF vs Normal_MEF ($n = 9$) (Fig. 3)

The ANOVA showed that there was a significant decrease of 8.6 dB in RWM velocity when the middle was filled with fat ($F(1,7) = 47.386$, $p < 0.0001$). There was also a significant effect of frequency ($F(2.281,15.965) = 23.355$, $p <$