

Cochlear Mechanics

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Frequency-Dependent Shear Properties of the Tectorial Membrane

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The vertebrate sense of hearing relies on a series of mechanical processes that deflect the sensory bundles of hair cells in the cochlea. Overlying these bundles is an acellular gel called the tectorial membrane (TM), which is believed to play a critical mechanical role in cochlear function. A variety of recent studies have shown that genetic changes to proteins found in the TM, such as α -tectorin (Legan et al., 2000, Legan et al., 2005), β -tectorin (Russell et al., 2007), and collagen type XI (McGuirt et al., 1999), lead to significant hearing loss. These studies show a correlation between TM molecular structure and cochlear function. However, the mechanical interaction of the TM with other cochlear structures, particularly the hair bundles of hair cells, remains unclear.

The TM is located in close proximity to the mechanically sensitive bundles of hair cells. Since these bundles are sensitive to shearing deflection, the response of the TM to shearing forces is of significant interest to cochlear mechanics. We have recently shown that radial forces applied to one location on a freely suspended TM launch traveling waves that propagate longitudinally along the TM (Ghaffari et al., 2007). These waves may contribute significantly to cochlear tuning and sensitivity, if they can be excited *in vivo*; that is, if OHC hair bundles and cochlear fluids displace the bulk of the TM rather than causing internal shear.

The shear impedance of the TM is a critical factor in determining whether waves can be excited, as well as the velocity and extent of wave propagation. The wave measurements were fit with a finite-element model that predicted a shear modulus that is significantly larger than most other estimates of that property. However, the results of these previous studies (reviewed in Richter et al., 2007) are not easily applied to the wave measurements. Many of these studies were performed on species that have a much lower range of best frequencies than the mice used for the wave measurements. Moreover, the other studies generally applied transverse forces at frequencies at or below 10 Hz, while the wave measurements applied radial forces in the 1-20 kHz frequency range.

To measure the shear properties of the TM, we developed a microfabricated probe that consists of a large base coupled to a shearing plate by a pair of cantilever arms (see Figure 1). Forces applied to the base were coupled to the plate through these arms. When the plate was brought into contact with the TM, some of this force was transmitted to the TM while some deflected the cantilever arms. The amount of deflection -- that is, the relative motion of the shearing plate and the base -- depended on the relative impedance of the TM and the cantilever arms. The cantilever arms could also be deflected laterally, so the probe was used to measure impedance in two dimensions.

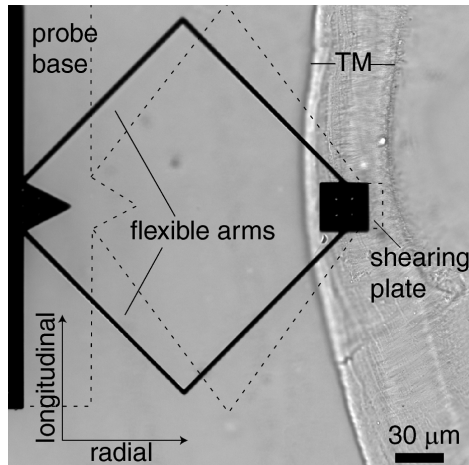


Figure 1: Microfabricated probe on a TM specimen. The probe consists of a base and shearing plate, connected by two flexible arms. The probe is capable of applying force in the radial and longitudinal directions, indicated by the arrows in the lower left hand corner. The radial fibrillar structure of the TM is readily visible in the image and was used to align the radial and longitudinal axes, the radial axis being parallel to the radial fibrils.

Figure 2 shows measurements of the TM impedance versus frequency for five TM specimens. For reference, the figure also shows the frequency response of a pure elastic spring and a pure viscous damper. The slope of the least-squares fit power-law relation of impedance magnitude with frequency was -16 ± 0.4 dB/decade for radial forces and -19 ± 0.4 dB/decade for longitudinal forces. These slopes did not show significant variations with frequency for forces in either direction. Both values were less than the slope of -20 dB/decade expected for purely elastic materials, but greater than the slope of 0 expected for purely viscous materials. The magnitude and phase values were tightly clustered in a narrow range. At 10 Hz, the magnitudes of the least-squares power law fits to impedance were 63 ± 28 mN·s/m in the radial direction and 36 ± 9 mN·s/m in the longitudinal direction. No obvious correlation between impedance magnitude and TM width was seen, although the range of TM widths in this study was small. For individual TMs, the impedance at 10 Hz was 1.7 ± 0.4 times larger in the radial than the longitudinal direction. The phase of impedance was nearly constant at $-72 \pm 6^\circ$ for radial forces and $-78 \pm 4^\circ$ for longitudinal forces at frequencies below 5 kHz. For longitudinal forces, the phase increased somewhat at the highest frequencies, consistent with the measured effects of the fluidic boundary layer.

The TM shear impedance measurements indicate that the TM is mostly elastic, with the viscous component of impedance at least three times smaller at all frequencies in both the radial and longitudinal directions. The magnitudes of both the elastic and viscous components of TM shear impedance decreased with frequency. In the longitudinal direction this decrease was nearly linear, but in the radial direction the magnitude decreased significantly slower than linearly with frequency. In other words, the effective stiffness of the TM increased with frequency while the effective viscosity decreased. The radial stiffness increased by roughly a factor of two with each decade in frequency, while the radial damping decreased by about a factor of five. Since our measurements were made at (nearly) constant displacement, the frequency-dependent stiffness might seem consistent with strain-rate-dependent impedance, e.g. strain hardening of collagen fibrils. However, shear impedance was nearly constant or decreased only slightly with increasing force at a given frequency (data not shown), so the changes with frequency are not likely to reflect a strain-rate dependence. This distinction is significant because it shows that the TM responds at low force levels as a Newtonian material (i.e., level-independent impedance) with frequency-dependent impedance.

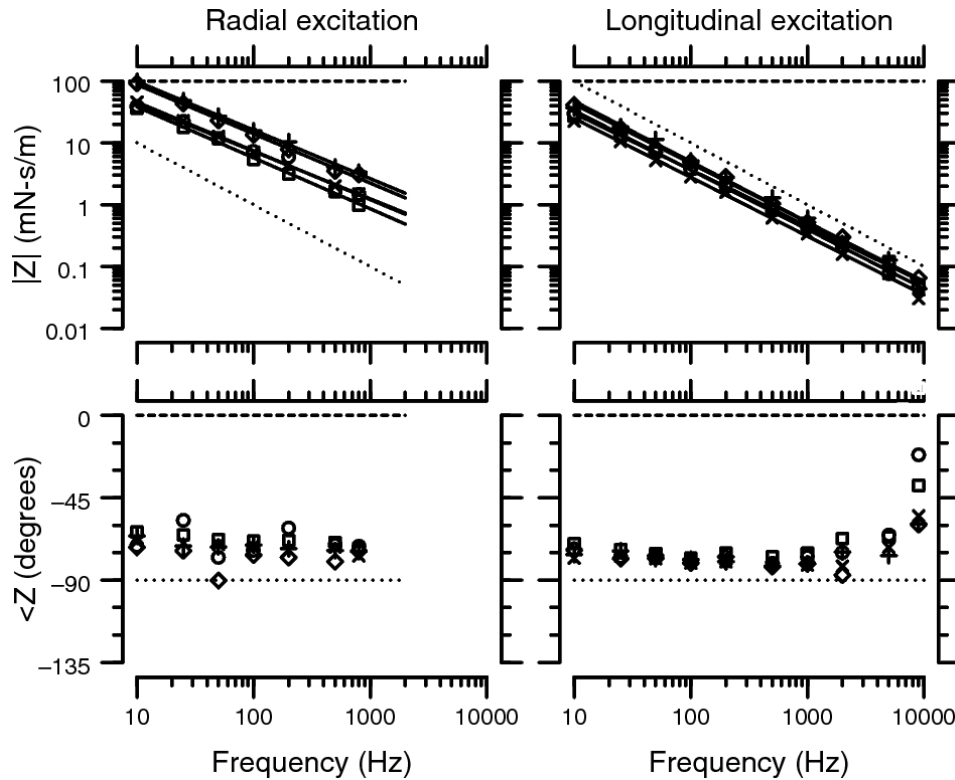


Figure 2: TM impedance versus frequency. The magnitude (top) and phase (bottom) of TM shear impedance are plotted vs. frequency for both radial (left) and longitudinal (right) forces. The lines represent least-squares power-law fits to measurements from individual TMs. The slopes of these power-law relations were -16 ± 0.4 dB/decade and -19 ± 0.4 dB/decade for radial and longitudinal forces, respectively. The dotted and dashed lines represent the frequency response of a pure elastic spring (slope -20 dB/decade and phase -90°) and a pure viscous damper (slope 0 and phase 0°), respectively.

Col11a2 Deletion Reveals the Molecular Basis for TM Mechanical Anisotropy

The TM has a significantly larger stiffness in the radial direction than other directions: a prominent mechanical anisotropy that is believed to be critical for the proper functioning of the cochlea. To determine the molecular basis of this anisotropy, we measured material properties of TMs from mice with a targeted deletion of *Col11a2*, which encodes for collagen XI. *Col11a2*^{-/-} mice have a 40-50 dB hearing loss, and have disorganized TM collagen in an otherwise morphologically normal cochlea. Figure 3 shows light micrographs of TMs from wild-type and *Col11a2*^{-/-} mice. Several prominent structures are visible in both images. Hensen's stripe, which is located adjacent to the hair bundles of inner hair cells, appeared unaffected by the *Col11a2* disruption. The radial fibrillar structure of the TM was still visible in *Col11a2*^{-/-} TMs. However, the density of radial collagen fibers decreased. In images of wild-type TMs, the brightness of the fibers cycled between light and dark roughly every $2 \mu\text{m}$. In *Col11a2*^{-/-} TMs, this modulation spanned a distance of about $4 \mu\text{m}$. Thus the deletion appeared to reduce the density of radial fibers.

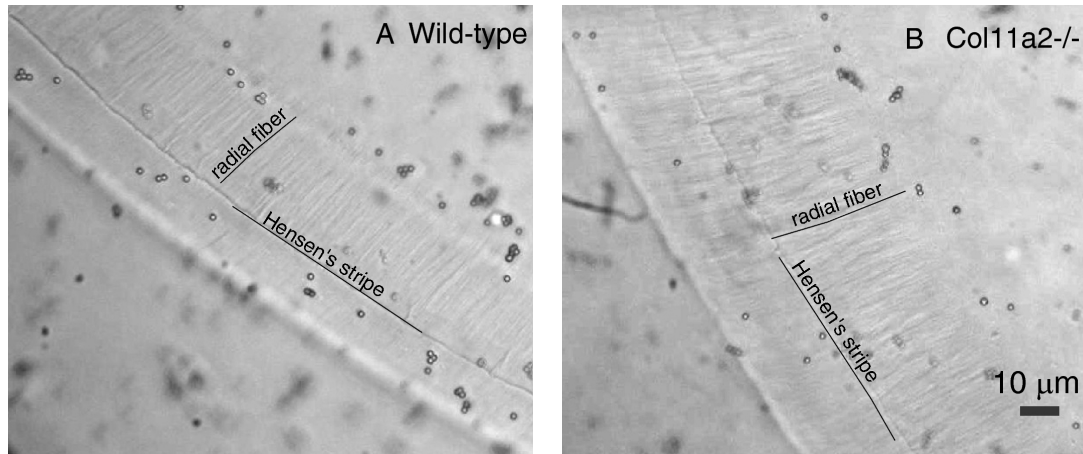


Figure 3: Light micrographs of TMs from (A) wild-type and (B) Col11a2^{-/-} mice taken using brightfield imaging. The TM extends from the upper left to lower right of each image. Radial fibrils and Hensen's stripe are visible in both images. However, the density of radial fibrils is lower in the Col11a2^{-/-} TM. The circles scattered throughout the images are microbeads used as markers.

Because the collagen fibers that were disrupted in Col11a2^{-/-} mice are oriented primarily in the radial direction, the Col11a2 disruption was expected to preferentially affect the radial shear impedance of the TM. To evaluate this prediction, measurements of shear impedance in both the radial and longitudinal directions were made at multiple frequencies using a microfabricated probe (described above). Figure 4 summarizes the magnitude and phase of shear impedance for TM segments from wild-type and Col11a2^{-/-} mice. The magnitudes of the shear impedance for the TMs from Col11a2^{-/-} mice ($n=3$) were 5.5 ± 0.8 dB lower than wild-types ($n=5$) in the radial direction averaged across all frequencies measured. In the longitudinal direction, shear impedance magnitude was 3.3 ± 0.3 dB lower for Col11a2^{-/-} mice than for wild-types. The ratio of radial to longitudinal impedance at 10 Hz was 1.0 ± 0.1 for Col11a2^{-/-} TMs versus 1.8 ± 0.7 for wild-type TMs. Thus the Col11a2 deletion eliminated the anisotropy of TM shear impedance. The slopes of shear impedance magnitude versus frequency for wild-type TMs were 16 ± 0.4 dB/decade for radial forces and 19 ± 0.5 dB/decade for longitudinal forces. For Col11a2^{-/-} TMs, these slopes were 16 ± 1.0 dB/decade and 19 ± 0.8 dB/decade, respectively. The phases of shear impedance for wild-type TMs were $-72 \pm 6^\circ$ for radial forces and $-74 \pm 11^\circ$ for longitudinal forces. For Col11a2 TMs, these phases were $-74 \pm 4^\circ$ and $-72 \pm 10^\circ$, respectively. Neither the slopes nor the phases differed significantly between wild-type and Col11a2^{-/-} TMs. These results indicate that the deletion reduced the magnitude of radial shear impedance without altering proportions attributed to elastic and viscous interactions.

Among hair cell organs, the presence of collagen in the overlying gelatinous matrix is unique to the mammalian TM (Thalman et al., 1985, Richardson et al., 1987). A computational model of TM material properties suggests that the radial stiffness of the TM is dominated by these collagen fibrils (Gavara and Chadwick, 2009}. Moreover, radial variations in TM shear modulus are correlated with the local density of collagen fibrils (Shoelson et al., 2004). Figure 4 shows that the Col11a2 deletion eliminated the difference between radial and longitudinal shear impedance, demonstrating that the mechanical anisotropy of the TM is due to collagen. However, collagen type XI makes up only a small fraction of the total collagen in the TM. Thus the effect on TM shear impedance of a targeted disruption in collagen XI is most likely an indirect one.

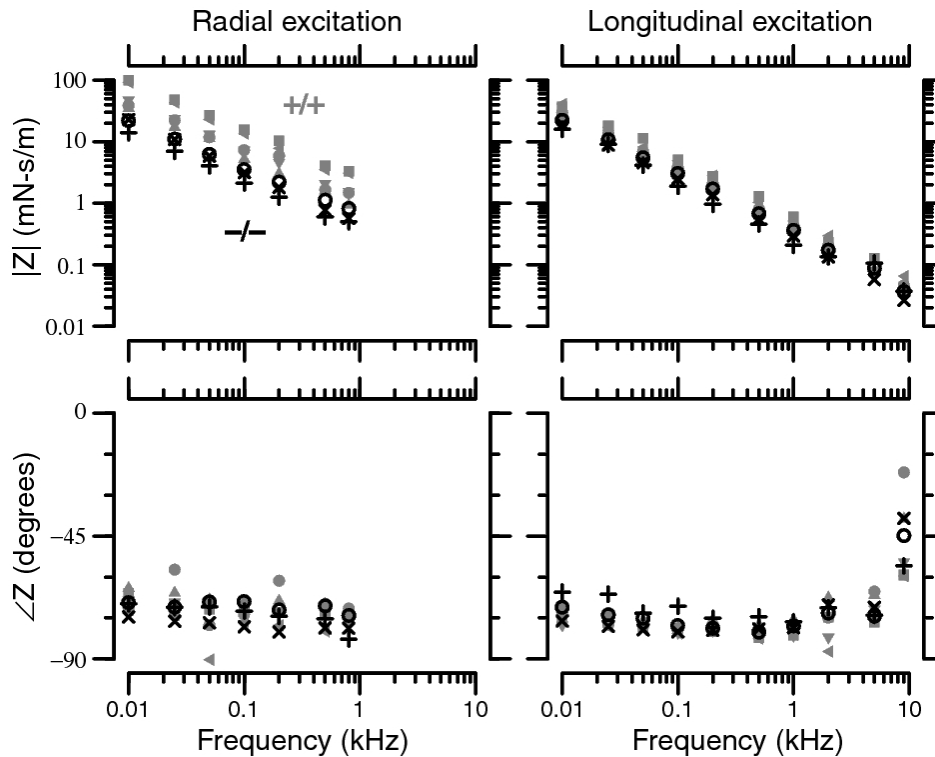


Figure 4: Magnitude (top) and phase (bottom) of shear impedance as a function of frequency, for both radial (left) and longitudinal (right) excitation. The plot symbols represent individual measurements from wild-type ($n=5$, grey symbols) and Col11a2^{-/-} ($n=3$, black symbols) TMs. Grey symbols are from figure 2.

In cartilage, collagen type XI is thought to be important for maintaining the interfibrillar spacing and fibril diameter of collagen type II (Mendler et al., 1989, Eikenberry et al., 1992, Li et al., 1995). Therefore, we expect deletion of collagen type XI to affect the organization and strength of the radial fibers in the TM. This argument is supported by electron micrographs which show loss of organization of the collagen fibrils in Col11a2 knock-out mice (McGuirt et al., 1999). That is, the elimination of anisotropy is due not to the absence of collagen itself, but to a change in its organization into fibrils. This conclusion is supported by the fact that the longitudinal shear impedance also decreased, but by a lesser amount.

Perhaps the most surprising finding was that the radial shear impedance decreased by only a factor of two. This finding, and the fact that shear impedance is only twice as large radially as longitudinally in wild-type TMs, suggests that the radial collagen fibrils are an important contributor to TM shear impedance, but not the only one. It is possible that the disorganized collagen fibrils continue to provide some resistance to shear. Other material components such as the striated sheet matrix may also contribute to shear impedance.

Publications

Journal Articles, Published

Gu, J. W., W. Hemmert, D. M. Freeman, and A. J. Aranyosi, "Frequency-dependent shear impedance of the tectorial membrane," *Biophys J* **95**:1529-38, 2008.

Masaki, K., J. W. Gu, R. Ghaffari, G. Chan, R. J. H. Smith, D. M. Freeman, and A. J. Aranyosi, "Col11a2 Deletion Reveals the Molecular Basis for Tectorial Membrane mechanical Anisotropy," *Biophys J* **96**: 4717-24, 2009.