Multiscale 3D organization of human auditory ossicles unveiled by synchrotron small-angle X-ray scattering tensor tomography

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Background
The transmission of sound through the human middle ear is a complex process that relies on the biomechanical properties of the ossicular chain, comprising the malleus (hammer), incus (anvil), and stapes (stirrup). These ossicles are the smallest bones in the human body and are responsible for amplifying and efficiently transmitting sound vibrations from the tympanic membrane to the cochlea [1]. Their biomechanical properties are intricately linked to their structure, spanning from the molecular scale (e.g., collagen triple helix) to the millimetric scale (e.g., foramen).

In cases of conductive hearing loss resulting from an impaired ossicular chain, reconstructive surgeries are necessary to restore hearing function. In these procedures, the incus plays a crucial role as either a support for prostheses or as a material to be sculpted itself, serving as an autologous passive implant [2]. However, the optimal positioning of prostheses on the incus and the effects of the carving process on incus biomechanics remain largely unknown and would strongly benefit from an improved knowledge of the ossicles' organization across several length scales.

The ossicles display a hierarchical bone organization, beginning with collagen triple helices that further assemble into fibrils with a periodic d-spacing of approximately 67 nm. These fibrils are mineralized by hydroxyapatite (HA) forming mineral platelets that mostly align with collagen fibrils. However, the shape of these nano-mineral particles and the orientation of collagen fibrils across a significant volume of ossicle, as well as their interactions with the vascular and nutritional foramen network, remain largely unclear to date.

Recently developed Small-Angle X-ray Scattering Tensor Tomography (SAS-TT) has emerged as the preferred method for 3D nanostructural characterization of mineralized tissues like bone over extended spatial regions. SAS-TT combines principles from Small-Angle X-ray Scattering (SAXS), which provides insights into anisotropic non-crystalline structures at sub-um scales, with X-ray tomography, enabling 3D volume measurements. The complete 3D reciprocal space (scattering directions) is reconstructed for each voxel, leading to detailed information about the 3D main orientation and degree of orientation of the set of nano-structures within each voxel.

In this study, our objective is to elucidate the organization of nano- and micro-structures within the human incus, clinically the most crucial auditory ossicles in surgery, using SAS-TT. We aim to gain insights into the preferential orientations induced by sound propagation and identify potential sites of bone remodeling. Additionally, we aim to understand the effect of the sculpting process and erosive pathologies on the inner organization of the incus body.

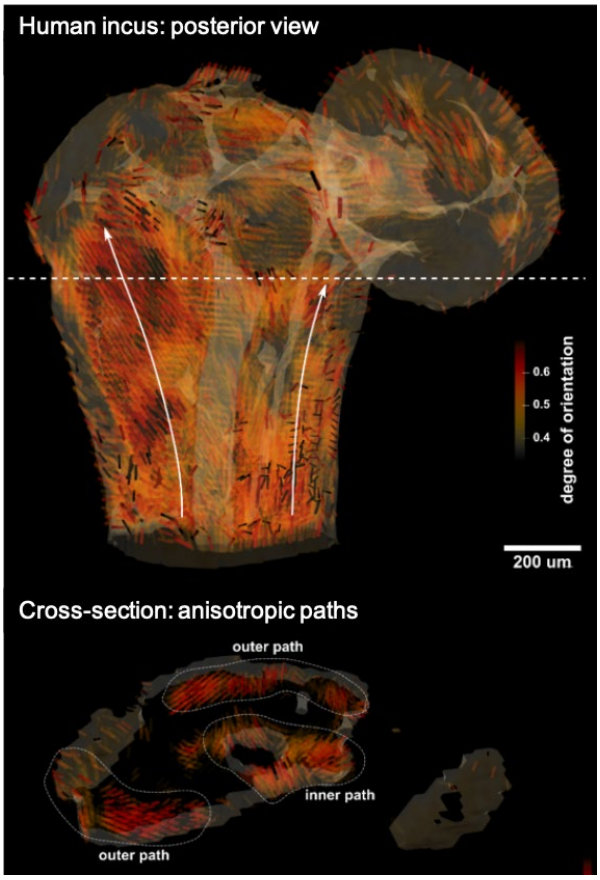
Methods
Our study includes 5 human incudes in various conditions: 3 healthy, 1 sculpted during ossiculoplasty, and 1 pathological with cholesteatoma, obtained from anonymous donors. The samples were further cut and milled down to a size of approximately 2.5 x 1.5 mm2 each. In the end, the long crus of 2 healthy incudes, and 3 sub-volumes from the body of 1 healthy incus, 1 sculpted incus, and 1 pathological incus, were examined using SAS-TT. The experiments were conducted at the PXI beamline of the SLS (Swiss Light Source, PSI), using a focused beam of 25 x 25 μm^2 at 12.4 keV and a 4M EIGER detector enabling acquisitions at 300 Hz, resolving a wide energy range. Detector data were reintegrated into 32 azimuthal bins in the range of q = 0.014 – 0.36 Å−1 and symmetrically averaged to eliminate detector gaps. The remaining 16 azimuthal bins were used as input for the SAS-TT reconstructions using the mumott Python library (https://mumott.org).

Results
First results were obtained on the long crus of 2 human incudes. The samples comprised the long process followed by the lenticular process that articulates with the stapes, the last bone of the ossicular chain. We were able to extract the main orientation of the mineralized collagen fibrils and their anisotropy, enabling the identification of 3 regions along the long process that exhibit a higher degree of orientation. These 3 regions with anisotropic collagen arrangement are located along the main vascular channel in the center of the long process, and converge at the bony pedicle, the junction between the long and the lenticular processes.

Further q-resolved analysis revealed the presence of three distinct phases corresponding to various shapes and sizes of mineralized particles within the incus, specifically distinguishing the inner part of the long process from the annular shell around the lenticular process. Subsequently, we conducted a comparison of 3 incus bodies in various conditions (healthy, pathological and sculpted). The distribution of orientations and anisotropy were extracted for each condition. While no significant differences were observed between the healthy and sculpted samples, the incus body affected by cholesteatoma exhibited a more isotropic collagen fibril organization, with a broader orientation distribution across the sample and lower local degrees of orientation. Additionally, we used the mean intensity as a measure of the mineral density to quantify the prevalence of low-density tissue in the pathological case, suggesting that bone remodeling occurred at different sites.

Conclusions
SAS-TT enables access to structural parameters at both nano- and micro-scales throughout the entire volume of samples. When applied to investigate human auditory ossicles, it revealed anisotropic pathways at the collagen fibril level, which may play a crucial role in guiding sound waves through the bone. Additionally, it identified regions with diverse nano-structuring, pinpointing sites of potential bone remodeling. Furthermore, it shed light on changes occurring in the bone in cases of erosive pathologies such as cholesteatoma. These findings could provide valuable insights for optimizing middle-ear surgery, with wide-ranging benefits for patients with conductive hearing loss.

Graphic:



Keywords:

X-ray Scattering, Tomography, Hearing Bones

Reference:

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