A NOVEL METHOD FOR THE 3D ASSESSMENT OF BONE ULTRASTRUCTURE ORIENTATION USING SAXS

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Introduction

Different orientations in bone ultrastructure determine bulk mechanical behavior and even more importantly the mechanical anisotropy of bone tissue at the local level [Reisinger, 2011]. Although finite element (FE) modeling is an established method for *in silico* biomechanical testing, FE models are typically assigned isotropic material properties disregarding this local bone anisotropy. This is mostly due to the fact that there is no method available that allows measuring ultrastructural bone anisotropy in 3D. Currently, small angle X-ray scattering (SAXS) offers the possibility to assess bone ultrastructure orientation in 2D [Fratzl, 1999]. Here, we present a novel method to derive and spatially resolve the 3D orientation of mineralized collagen fibrils in bone using scanning SAXS.

Methods

A new mathematical expression to obtain the 3D fibril orientation from 2D alignment information was derived and tested using SAXS experimental data. A trabecular bone specimen from a human vertebral body (Fig. 1A) was embedded and sectioned in 20 µm slices (Fig. 1B). Scanning small angle X-ray scattering (sSAXS) experiments were carried out at the cSAXS beamline of the Swiss Light Source in rotation steps of 10°, over an angular range of 360° (Fig. 1C). A range of 60 degrees, where slices were close to parallel to the X-ray beam, was excluded. For each angular position the sample was raster scanned with a 20 µm step size. SAXS power spectra for an area of 60×95 points (Fig. 1B, red box) were recorded. For each point, the mean 2D fibril orientation angle χ (Fig. 1D) was derived according to [Bunk, 2009]. The polar and azimuth angles (θ, φ) that describe the 3D fibril orientation (Fig. 1E) were obtained for each point by fitting the acquired 2D orientations χ for all rotation angles ω to the new formula (Fig. 1F).

Results and Discussion

Experiments were well described by the new formula (Fig. 1F). The 3D fibril orientation for every point of the assessed area was derived. 93.5% of all points exhibited a clearly defined fibril orientation (R^2 > 70%, Fig. 1F), while transition points between differently oriented domains accounted for 6.5% of all points.

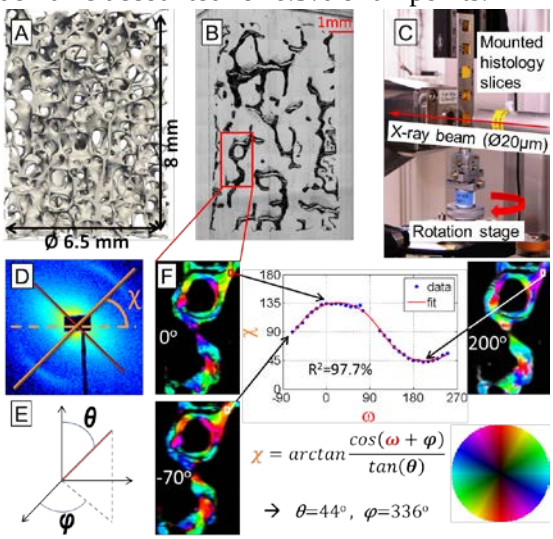

*Figure 1. (**A**) Micro-computed tomographic reconstruction of the studied vertebral specimen. (**B**) Light microscopic image of a histological section. (**C**) Histological sections mounted on a rotation stage for sSAXS. (**D**) SAXS power spectrum, from which mean 2D fibril orientation (χ) was derived. (**E**) The polar and azimuth angles (θ, φ) used to describe the 3D fibril orientation. (**F**) Spatially resolved, color-coded 2D orientation (χ) for three different rotation angles (ω). The angle (χ) is given by the pseudo-color map (bottom right). In the middle, experimental data and fit for a specific point of the structure, and the new formula to derive 3D orientation angles θ and φ (dots: experimental data from sSAXS, continuous line: new method to derive fibril orientation in 3D).*

Conclusions

Using a novel mathematical and experimental approach, we derived the 3D orientation of mineralized fibrils in human bone in a spatially resolved manner using sSAXS. Access to 3D bone ultrastructure will allow FE modeling of bone function including local bone anisotropy.

References

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