Probing the micro-mechanical behavior of bone via high-energy x-rays

J. Almer,[1] S.R. Stock[2]
[1]*Argonne National Laboratory, Argonne, IL U.S.A.;* [2]*Northwestern University, Chicago, IL, U.S.A.*

Introduction

Bone is a highly-adaptive, particulate-reinforced composite which, through a complex hierarchical structure, achieves excellent mechanical performance. The composite preserves, to a large degree, the desirable properties of the individual components: high toughness of the bone matrix, collagen fibrils stabilized by water, and high stiffness of the reinforcing phase, nano-sized crystallites of carbonated apatite. Understanding bone fragility (osteoporosis) requires quantifying mechanical input to bone and identifying "weak-link" microstructures. This mechanical input has been quantified *in vivo* with strain gages attached to cortical bone, but attached strain gages do not probe subsurface mechanical response.

We addressed this shortcoming recently by appling wide- and small-angle x-ray scattering to canine fibula sections, to study the micro-mechanical response of bone on different length scales [1]. These data provide a unique view of load partition between the constituent phases of bone, and here we extend these measurements to an entire rat tibia, where strain gradients due to bending are anticipated.

Methods and Materials

Tibiae of 14 week old Sprague-Dawley rats were studied. A 3D microCT rendering of the sample and definitions of the loading (y) and transverse (x) directions appear in Fig.1, with the y-axis approximately parallel to the bone's longitudinal axis. Due to the curved shape of the tibia, significant sample bending in the x-direction was anticipated even under uniaxial compression, similar to that expected *in vivo* (there was little curvature in the y-z plane). The sample cross-section at y=0 was determined by microCT to be approximately 4 mm^2.

The sample was potted in epoxy and compressed in a load frame designed for in situ x-ray scattering studies. Loading was in displacement control, at a rate of 0.06 μm/sec. The aggregate macroscopic response was followed using a load cell combined with strain gages located on both the 'convex' (-x) and 'concave' (+x) sides of the sample. While under load, high-energy x-rays (80.7 keV) of transverse size 0.05(x) x 0.05(y) mm^2 were used to sample through the entire thickness (z) of the sample. Wide-angle scattering patterns at multiple x-positions (y=0) were collected using a large area detector, with each 2D pattern containing data in a plane approximately parallel to the sample x-y plane.

Results

Internal strains along the longitudinal/loading direction (ε_{yy}) are shown for the apatite (002) reflection in Fig. 1. Values for five different lateral positions are shown, with x=-1 mm near the convex side of the sample and x=+1 near the concave side. Also shown are value from the strain gage located on the concave side of the specimen.

All internal strains are non-zero before unloading and ε_{yy} ~ -700 με. When stress is applied, strain response varies substantially across the sample, with ε_{yy} (x=1) showing the highest compression while ε_{yy} (x=-1) slightly more tensile values. The macroscopic strain increases similar to, but at a higher degree than, ε_{yy} (x=-1). At the maximum applied stress of ~33 MPa the sample experienced multiple cracks, as verified via post-mortem analysis. Upon unloading the macroscopic strain was primarily elastic, as values (nearly) returned to those seen upon loading.

Discussion

The presence of compressive residual strains ε_{yy} in the bone is consistent with [1] and appears to be strongly linked to sample hydration state [2]. While efforts were made to hydrate the rat tibia during experiments, some drying may have occurred and contributed, at least in part, to the observed residual strains.

The strain variations across the sample width indicate that the bone experienced considerable bending during loading, with the neutral axis at y=0 being at approximately x=0.75. The macroscopic strain trends from the strain gages (including the concave gage, not presented here) are qualitatively consistent with the gradients seen via x-rays. Higher values of macroscopic strain are expected based on the stiffer elastic properties of the mineral phase cf. the aggregate response, so the data in Fig.1 is quantitatively consistent as well.

This and future high-spatial resolution x-ray strain data, collected *in situ*, should be of considerable use as input to models (e.g. FEM) of deformation in complex bone shapes.

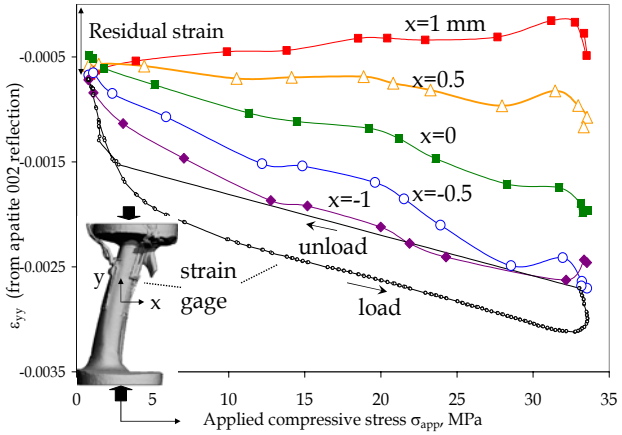

Fig. 1. Strain along the longitudinal (y) direction as a function of applied stress and position. All x-ray strains taken during loading, while strain gage data shown for both loading and unloading. Strain-gage data was offset by ε=-700με for clarity.

Acknowledgements

We thank Dr. K. Igarashi, Tohoku University, Japan for providing the rat tibiae. Use of the APS was supported by the Department of Energy, Office of Science, Office of Basic Energy Science, under contract No. W-31-109-Eng-38.

References

[1] J. Almer and S. Stock, J. Struct. Biol **152**, 14 (2005).
[2] J. Almer and S. Stock, J. Struct. Biol, submitted.