

# THE ROLE OF MINERAL HETEROGENEITY AND MATERIAL ANISOTROPY ON TRABECULAR BONE MECHANICS

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## Introduction

Trabecular bone has a multiscale structure, which exhibits a high level of heterogeneity and anisotropy. At the architectural scale, it features a complex network of trabeculae: small elements with different shapes, sizes, and orientations (Fig. 1a). At the tissue level, each trabecula is heterogeneous and composed of different bone packets (Fig. 1b-c), resulting from the combined processes of bone remodeling and mineralization [1]. Each bone packet has a unique shape, distinct mineral content and orientation of mineralized collagen fibrils. The role of bone packets on the mechanical behavior of individual trabeculae and of trabecular networks however is still not clear, although bone packets are assumed to influence damage behavior [2] and may be important for the material quality of trabecular bone [3]. Here, we characterized the mineral content of bone packets using quantitative backscattered electron imaging (qBEI). Their mechanical behavior was analyzed by nanoindentation (nIND). We addressed anisotropy by choosing, with the help of micro-computed tomography (micro-CT), trabeculae parallel and perpendicular to the analyzed surface.

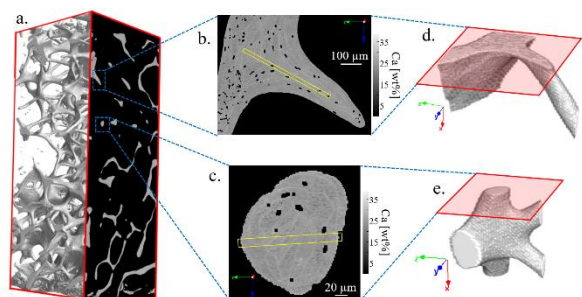


Fig. 1: (a) Combination of qBEI and micro-CT on the bone sample. (b-c) Magnified qBEI maps of selected trabeculae, the yellow box highlights the indented regions. (d-e) Micro-CT reconstructions of selected trabeculae below the exposed surface.

## Methods

Trabecular bone was extracted from iliac bone autopsy sample of a healthy 93 y.o. woman. qBEI was performed with a scanning electron microscope (20 kV voltage, 10 mm working distance, calibration with carbon and aluminum, 1.7  $\mu\text{m}$  pixel size) to obtain 2-dimensional maps of calcium content on the exposed cross-section. The sample was then imaged with micro-CT (80 kV voltage, 4.3 voxel size) to acquire 3-dimensional information on the trabecular network below the exposed surface. By superimposing the two techniques, trabeculae oriented roughly parallel and perpendicular

to the qBEI plane could be selected (Fig. 1d-e). Reduced modulus ( $E_r$ ) and hardness ( $H$ ) were then measured on the selected trabeculae, carefully choosing regions that crossed different bone packets, using nIND (Berkovich tip, 2000  $\mu\text{N}$  load, 2.5 - 5  $\mu\text{m}$  spacing between indents). Mineral content and mechanical properties were correlated by superimposing qBEI and nIND maps.

## Results

Mineral content and reduced modulus profiles across two trabeculae are shown in Fig. 2. The mean  $E_r$  in the parallel trabecula was approximately 14% lower compared to perpendicular one, whereas the difference in mean Ca content was only 1%. Of particular interest is the lower trend of  $E_r$  observed in a region of the parallel trabecula, which cannot be explained by the Ca profile only.

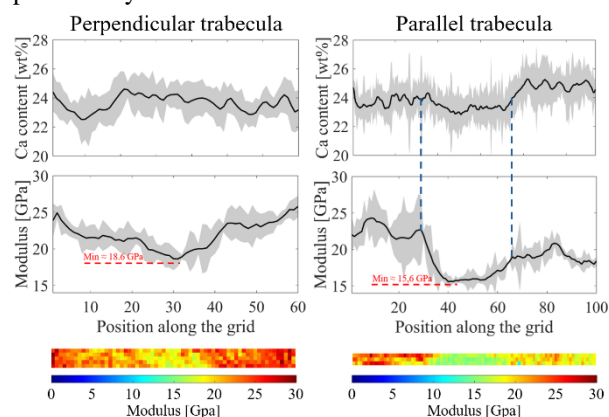


Fig. 2: Spatial evolution of Ca content and  $E_r$  across two trabeculae, with corresponding  $E_r$  maps. The dotted red lines indicate the minimum values of  $E_r$ , while the dotted blue lines highlight the decreasing trend of  $E_r$ .

## Discussion

By comparing the mineral content and mechanical properties at the same locations on two trabeculae having different orientations, we found practically no differences in mineral content but dissimilar elastic properties. We assume that material anisotropy, due to the underlying orientation of mineralized collagen fibrils within the lamellae of the bone packets, may contribute to such different mechanical response. This is currently under investigation using second harmonic generation (SHG) imaging. Our findings may be cast into future computational models of trabecular bone.

## References

1. Ruffoni et al., Bone 40, 2007
2. Smith et al., Journal of Biomechanics 43, 2010
3. Busse et al., Bone 45, 2009

