MINERAL CONTENT AND BIOMECHANICAL PROPERTIES OF FIBROLAMELLAR BONE

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Introduction

Fibrolamellar bone is a peculiar type of bone found in the long bones of young large mammals, which combines the conflicting requirements of a fast growth with high stiffness and strength, as required to withstand the weight of such large animals [1]. Fast growth is achieved by depositing a primary hypercalcified layer (PHL) of disordered bone, located in between two blood vessels. This central layer acts as a scaffold to direct the formation of parallel-fibered bone (PFB), having mineralized collagen fibers well oriented along the longitudinal direction of the bone. When reaching the blood vessels, PFB is then replaced by lamellar bone (LB), which features a few alternating bone lamellae with collagen fibers oriented in different directions. A so-called fibrolamellar bone unit (FBU) comprises these three different types of bone. Although the microstructure of fibrolamellar bone is known [1], the corresponding biomechanical properties are not well characterized. Specifically, there is no information about the local mechanical behavior of each basic building block. Yet, this tissue is of clinical relevance given the similarity between its deposition and callus formation in bone healing. Here, the correlation between local mechanical properties and mineral content of fibrolamellar bone is investigated by combining standard nanoindentation (nIND), nanoscale modulus mapping (nanoMM) and backscattered electron imaging (BEI), including quantitative analysis (qBEI).

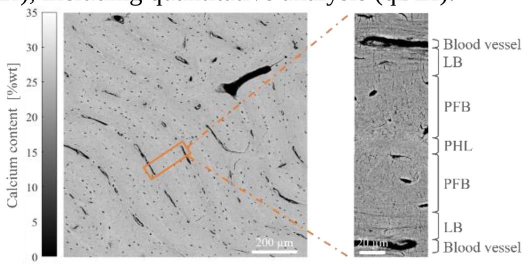

Fig. 1: 2D map of mineral content along with a higher magnification backscattered electron image of a FBU.

Methods

Samples from two femurs of calves were harvested and prepared, considering both longitudinal and transverse sections. A combination of qBEI (1.8 µm pixel size) and BEI at higher magnification (57 nm pixel size) was used to quantify mineral content and highlight nanostructural features. Mechanical properties were measured across FBU using nIND (Berkovitch tip, 6 µm spacing between indents, 3000 µN) and nanoMM. The latter is based on applying a small static force (1 µN) to ensure elastic contact between tip and sample surface; a periodic modulation force (0.6 µN, 275 Hz) is then added to probe local storage modulus without plastically deforming the sample. In the present setting, this approach has a lateral resolution of about 80 nm [2].

Results

Quantitative analysis (Fig. 1) revealed a homogeneous mineral composition across the FBU with the exception of the PHL, which appeared more mineralized. In LB, brighter lamellae can be distinguished from darker ones with high resolution BEI, this contrast being due to different fiber orientation. Indentation modulus profiles highlighted stiffer bone within PFB and lower values within the PHL (Fig. 2). Higher resolution is needed to characterize submicron mechanical behavior within LB. NanoMM showed alternating storage moduli: thicker (2.56±0.24 µm), brighter lamellae are more than 1.5 times stiffer than thinner (2.03±0.43 µm), darker ones. As they have approximately the same mineral content, this difference can probably be explained by the different collagen orientation in neighboring lamellae.

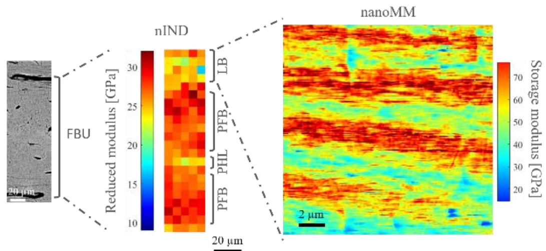

Fig.2: Mechanical properties across a FBU using nIND and nanoMM.

Discussion

We characterized the mechanical heterogeneity of fibrolamellar bone and showed that the PHL, being deposited very fast, has lower stiffness although having a higher mineral content compared to LB and PFB, therefore highlighting the critical role of collagen organization. The weak region is then reinforced by high stiffness PFB. High resolution nanoMM provided sub-micrometer biomechanical information which is not accessible with traditional nanoindentation. Yet, these data are relevant to reach a comprehensive understanding of the structure-function relationship of bone. Indeed, LB shows lamellae with a strong elastic contrast (higher than previously reported in osteonal bone) which may be needed to hamper crack propagation in the presence of only a few lamellae.

References

1. Almany Magal et al, J Struct Biol, 186:253-264, 2014.
2. Zlotnikov et al, Prog Mater Sci, 87:292-320, 2017.