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Biomechanical characterization of fibrolamellar bone using nano-scale modulus mapping

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Bone has the amazing ability to adapt its mass, structure and material properties to fulfill specific loading requirements. The bone of large and fast growing animals, such as cows and horses, has to solve two challenges: it has to grow very quickly and, at the same time, it has to provide sufficiently high stiffness and toughness. In general, the hierarchical structure of bone shows several strategies, at different length scales, to combine high stiffness with high toughness. This is particularly true for secondary lamellar bone, which is a type of bone formed rather slowly during the biological process of bone remodeling. The mechanical competence of fast growing bone is usually inferior to secondary bone, however nature has found other strategies to reinforce fast growing bone, also called fibrolamellar bone, such that it can sustain the high loads as required in large animals while allowing a rapid growth.

Fibrolamellar bone has received only limited attention, nevertheless it is a very relevant type of bone to better understand the rapid bone formation, as required, for instance during bone healing. The basic unit of fibrolamellar bone is formed between two large blood vessels. Firstly, a layer of hypercalcified disordered bone is quickly deposited. This serves, most likely, as a scaffold for the formation of parallel fibers bone, with the mineralized collagen fibers well oriented along the longitudinal direction. Around the blood vessels, parallel fiber bone is replaced by more classical lamellar bone. Although the structure of fibrolamellar bone is quite well-known, its mechanical properties are largely unexplored. Specifically, there is no information on the local mechanical behavior of the different basic blocks (i.e., hypercalcified layer, parallel fibers and lamellar region) which constitute fibrolamellar bone.

This master thesis proposes to use a complex and fairly new nanoscopic mechanical characterization method based on nanoindentation and called modulus mapping, to analyze with high spatial resolution the mechanical properties of a fibrolamellar bone unit. The modulus mapping technique was first applied to a standard fused quartz sample to tune the parameters and to establish a proper protocol to obtain reproducible data. This material has a know Young's modulus, allowing to calibrate the modulus mapping approach before going to bone.

In the second part of the thesis, fibrolamellar bone was investigated. Firstly, scanning electron microscopy was used to characterize the microstructure of fibrolamellar bone, confirming the different building units, although the mineral content of the central layer was not higher in comparison to other regions. Second, the local mechanical properties were investigated by combining traditional static indentations and modulus mapping. Interestingly, the central layer had a lower modulus then the other regions, suggesting a more disorganized arrangement of the mineralized collagen fibers. Additionally, the ratio of the elastic properties between thin and thick lamellae present in the lamellar region could be well characterized by modulus mapping and was much higher than traditionally reported in the literature. Furthermore, the parallel fiber region and the thick lamellae showed the highest elastic properties. Finally, high load indentation was used to generate local fractures and the fracture paths were characterized by electron microscopy. The results showed that fractures were deflected in the lamellar region around the large blood vessels while cracks could propagate relatively straight inside the parallel fibers region.

In conclusion, the present work reports, for the first time, local nanoscale elastic and fracture properties of fibrolamellar bone which are relevant to understand how nature combines the conflicting requirement of rapid bone growth with good mechanical properties. Future work may focus on the central layer to determined its composition using quantitative techniques such as qBEI and maybe apply this process to the unexplored cement line.

Keywords: fibrolamellar bone, scanning electron microscopy, nanoindentation, modulus mapping, storage modulus, high load indentation, fracture analysis.



Figure 1: Scheme of the different steps of the established protocol to realize a modulus mapping.



Figure 2: Results obtained with the classical method of static indentations. From left to right: Static indent grid made on a fibrolamellar bone unit and profile of the averages made on the thickness of the grid.



Figure 3: From left to right: Result obtain with modulus mapping for a lamellar bone region and high load indentation at the interface between lamellar bone and parallel fibers bone.