CEMENT LINES MATERIAL PROPERTIES AND INTERPLAY WITH THE LACUNO-CANALICULAR NETWORK IN HUMAN OSTEONAL BONE

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Introduction

Cement lines (CLs) are thin interphases connecting osteons to the surrounding bone. Biologically, CLs are formed during the reversal phase of bone remodeling and pioneer the formation of lamellar bone in secondary osteons [1]. Biomechanically, CLs are believed to protect osteons by deflecting microcracks. For this to happen, CLs should have different mechanical behavior than surrounding bone [2]. Despite their relevance, CL's properties are not well understood. Elucidating the interaction between CLs and the osteocyte lacuno-canalicular network (LCN) is of central importance in the context of bone mechanobiology. In previous work, we have shown that CLs not only have a higher mineral content than the corresponding osteons, but also that their degree of mineralization strongly depends on older adjacent bone [3], suggesting that resorbed bone may be locally recycled for the formation of CLs. In this study, we consider additional mechanical, material, and biological aspects of CLs. Using a multimodal approach with sub-micron resolution, as required by the tiny dimensions of CLs, we correlate different micro- and nanoscale characteristics on the same samples/locations to unravel the intricate structure-mechanics-function relationship of CLs in human bone.

Methods

We focused on femoral cortical bone samples extracted from two male donors (40 and 81 y.o.) and analyzed a total of 35 uninterrupted osteons. Elastic modulus and hardness were measured at various regions across the CLs using nanoindentation (nIND, 5500 indents). Quantitative and high-resolution backscattered electron imaging (qBEI & hrBEI) were combined to quantify mineral content and to locate the indents. To gain insight into the structural characteristics of mineral particles, synchrotron based small- and wide-angle X-ray scattering (SAXS/WAXS, ESRF, Grenoble) were used. Collagen orientation was visualized using second harmonic generation (SHG) imaging. Additionally, we analyzed the LCN using confocal laser scanning microscopy (CLSM) on rhodamine-stained samples.

Results

CLs exhibit higher mineral content, elastic modulus, and hardness compared to their corresponding osteons (Fig. 1 A-C). At the same time, CLs mineral particles were thinner and shorter than those found in the osteons (Fig. 1 D, F). A spatial mapping of the degree of crystal orientation shows a periodic pattern, corresponding to bone lamellae as identified by SHG. At the CLs, mineral was not particularly disorganized (Fig. 1E, G).

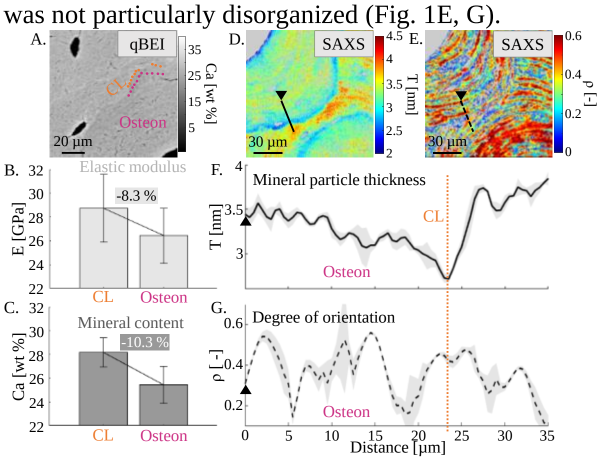

Fig. 1: Mechanical/mineral properties of CL/osteon.

Initial observations of the LCN indicate a reduced canalicular density at the border of the osteon, with some canaliculi penetrating the CL and connecting osteocytes from neighboring osteons (Fig. 2 red arrows).

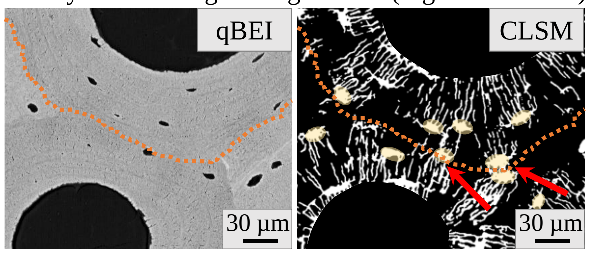

Fig. 2: Osteon-osteon connections across the CL.

Discussion

CLs exhibit mechanical, compositional, and structural differences compared to adjacent bone. The difference in mechanical properties was less than expected from the degree of mineralization, and this may be explained by smaller crystals. The mechanical contrast between CL and lamellar bone is modest, calling into question their presumed role on crack propagation. The demonstrated scarce osteon-osteon connections via the LCN are strongly related to the quality of bone mechanosensitivity, and it will be further explored through load-induced fluid flow simulations [4].

References

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