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Exploring Material, Mechanical and Biological Features of Cement Lines in Human Osteons

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Background: Cement lines (CLs) are thin interphases separating osteons from the surrounding bone. Despite their small size, CLs are believed to have a significant impact on bone quality. Biomechanically, CLs may contribute to bone fracture resistance by deflecting or stopping microcracks [Gustafsson et al. JBMMB, 90:556-565, 2019]. From a biological perspective, CLs precede lamellar bone formation in secondary osteons, and may facilitate osteoid deposition on the eroded surface [Lassen et al. JBMR, 32(7):1395-1405, 2017]. In bone mechanobiology, the relationship between CLs and the osteocyte lacunocanalicular network (LCN) is crucial. The number of canaliculi crossing CLs to connect osteocytes from nearby osteons should be related to the possible interaction between different osteons [Milovanovic et al. ACS Nano, 7(9):7542-51, 2013]. Despite their recognized importance, CLs remain among the least understood features of bones. In a previous work, we have shown that CLs have a higher mineral content than their corresponding osteons and that the degree of mineralization is strongly dependent on the mineral content of older adjacent bone [Cantamessa et al. Abstract for the 28th Congress of ESB, 2023], suggesting that the mineralization of CLs may rely on locally recycled bone. This study exploits a multimodal approach with sub-micron resolution to further investigate the mechanical, material, and biological properties of CLs in human osteonal bone.

Methods: Thirty-five uninterrupted osteons from femoral cortical bone samples extracted from two male donors (40 and 81 y.o.) were analyzed. Nanoindentation was employed to measure elastic modulus and hardness at various locations across the CLs (5500 indents). Quantitative and high-resolution backscattered electron imaging were combined to quantify mineral content and to precisely locate the indents. Synchrotron-based small- and wide-angle X-ray scattering (SAXS/WAXS, ESRF, Grenoble) were used to characterize the size, shape and orientation of mineral particles. Collagen orientation was visualized using second harmonic generation (SHG) imaging. The LCN was analyzed using confocal laser scanning microscopy on rhodamine-stained samples.

Results: CLs exhibit notable higher mineral content, elastic modulus, and hardness compared to their corresponding osteons. Mineral particles within CLs display a distinct morphology, appearing thinner and shorter than those within osteons (Fig. 1A). A spatially resolved analysis reveals a periodic pattern in crystal orientation, mirroring the lamellar organization identified by SHG. Remarkably, mineral orientation at the CL site is not particularly disorganized (Fig. 1B). Preliminary quantification of the LCN indicates reduced canalicular density along the osteon border, with only a few canaliculi extending into the CL region (Fig. 1C).

Conclusions: CLs possess distinct mechanical, compositional, and functional attributes compared to adjacent bone. Although stiffer than lamellar bone, the mechanical properties of CL are less than expected given their high mineral content, which may be due to the smaller crystal sizes. Moreover, the modest disparity in mechanical behavior between CLs and lamellar bone raises questions about the presumed role of CLs in mitigating crack propagation, as such processes usually require higher mechanical contrast. The sparse connections between osteons via the LCN question inter-osteonal communication through load-induced fluid flow.

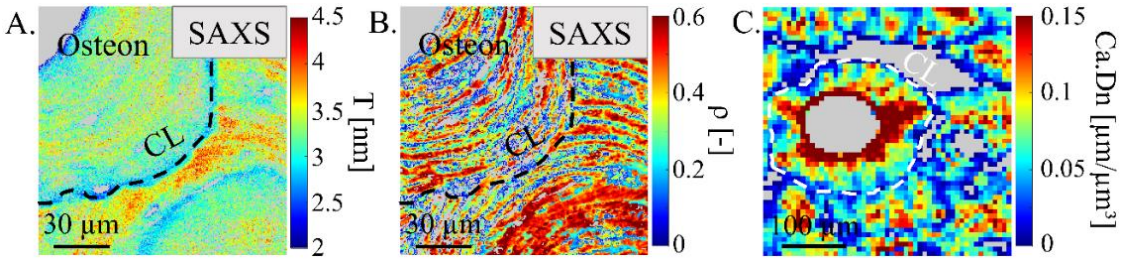

Fig. 1: (A) Mineral particle thickness and (B) crystal orientation of a portion of a representative osteon and its corresponding CL (a ρ of 0 means no predominant orientation and a ρ of 1 denotes perfectly aligned). (C) Canalicular density of an osteon and its corresponding CL.

Keywords: Cement lines, Bone material properties, Bone mechanobiology.