Exploring lacunocanalicular network specificity along the cement line in human osteons

L. Demeuldre[1*], A. Cantamessa[1*], S. Blouin[2], M. Rummler[3], A. Berzlanovich[4], R. Weinkamer[3], M. A. Hartmann[2], D. Ruffoni[1]

[1] University of Liège, Belgium; [2] Ludwig Boltzmann Institute of Osteology, Austria; [3] Max Planck Institute of Colloids and Interfaces, Germany; [4] Center of Forensic Medicine, Austria.

*These authors contributed equally to this work

Cement lines (CLs) are thin interphases separating osteons from the surrounding bone. They are deposited during bone remodeling, preceding the formation of the osteocyte lacunocanalicular network (LCN) in secondary osteons. Of central interest for bone mechanobiology is the possible interaction between the osteocytes inside an osteon and the cells of the surrounding environment. This interaction depends on the canaliculi crossing the CL and connecting to the osteocytes outside the osteon. However, the extent to which osteocytes can communicate across CLs remains debated: some studies reported a complete network disruption at CLs [1], while others found intact inter-osteon canalicular connections [2]. This work focuses on the characteristics of the LCN along the CLs. Human femoral cortical bone samples from two male individuals (40 and 81 y.o.) were obtained and rhodamine stained. The LCN was visualized using confocal laser scanning microscopy and analyzed using a dedicated software (TINA) [3]. Initial data revealed reduced canalicular density near the osteon boundary (Fig. 1A). The orientation of the canaliculi shifted from predominantly radial within the osteon to predominantly lateral at the outer periphery of the osteons (Fig. 1B). Visual examination of this region showed that most radially oriented canaliculi are interrupted at the CL. Yet, a few canaliculi could cross the CL. This is usually observed between adjacent osteons rather than between osteons and interstitial bone (Fig. 2A-D). While crossings exist, the limited occurrence suggests minimal inter-osteon communication, raising questions about the functional significance. Future analysis will explore how osteon mineral content (a surrogate of tissue age) may affect inter-osteon connectivity.

[1] Kerschnitzki et al., Journal of Structural Biology 2011; 173.2: 303-311.

[2] Milovanovic et al., ACS Nano 2013; 7.9: 7542-7551.

[3] TINA repository described in Repp et al., Bone Reports 2017; 6: 101-108.

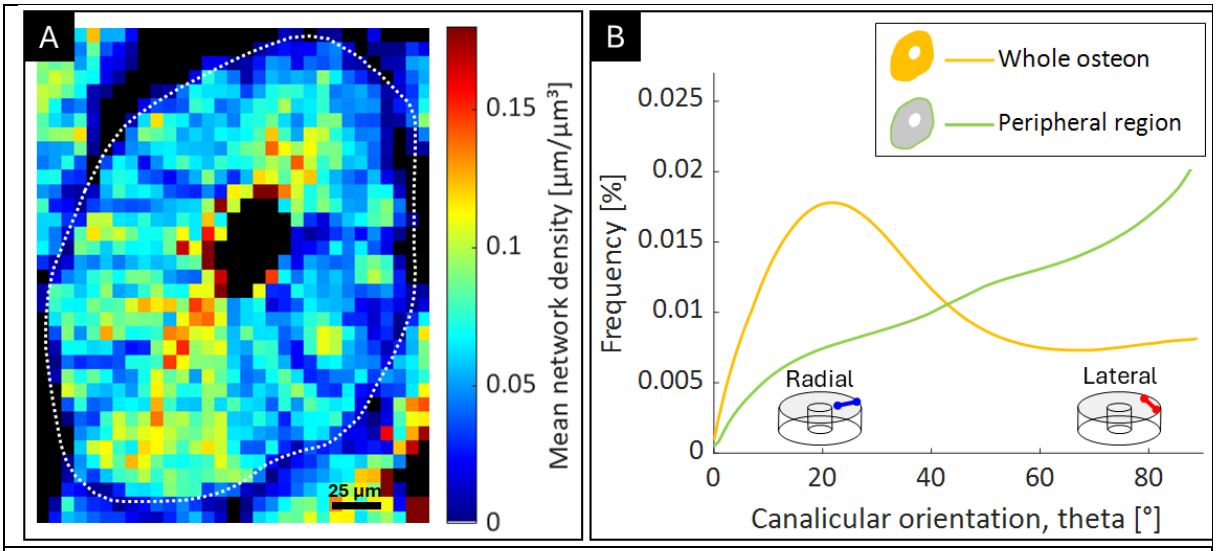

Figure 1. *(A) Mean density of the canalicular network with the CL (white dotted line) manually segmented. (B) Frequency distribution of canalicular orientation. Theta represents the angle between the radial direction and the direction of the canaliculi (theta = 0° for radially oriented canaliculi, and theta = 90° for laterally oriented canaliculi).*

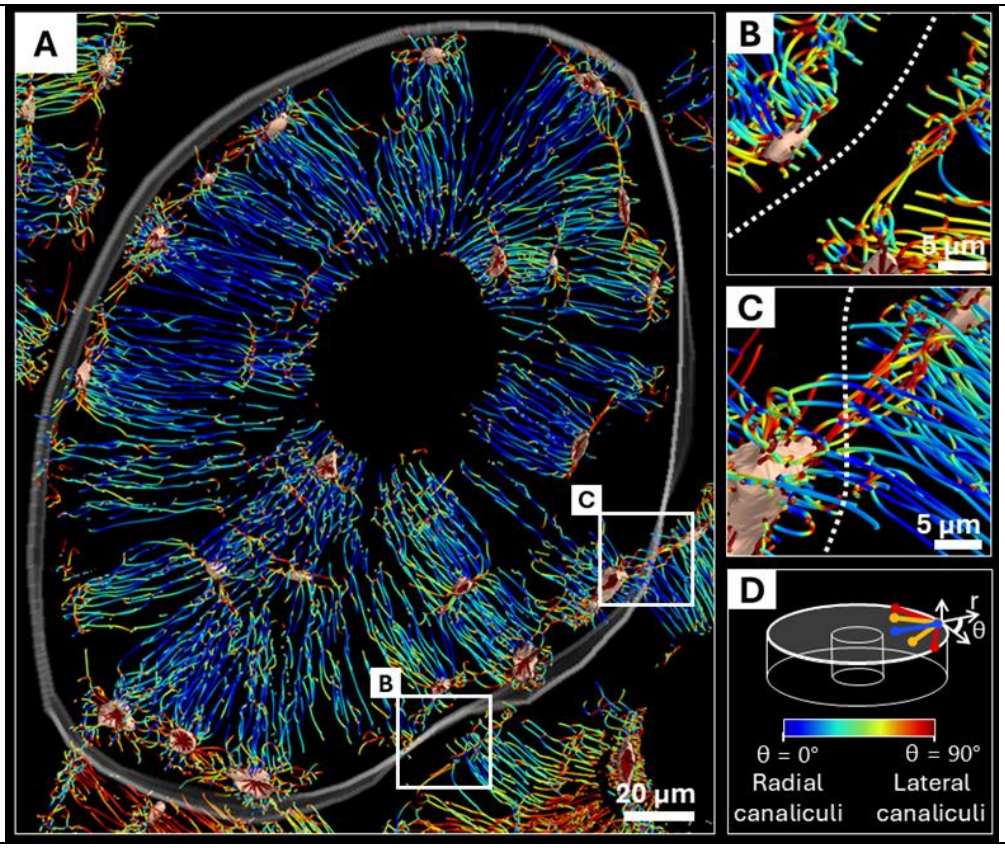

Figure 2. (A) *3D visualization of the LCN in a selected osteon, showing radially oriented canaliculi (blue), laterally oriented canaliculi (red), and lacunae (light brown). The CL is visualized as a grey layer bordering the osteon. Close-ups showing (B) canaliculi interrupted at the CL (white dotted line) and (C) multiple canaliculi crossing the CL. (D) Color bar showing canalicular orientation.*