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Characterization of the osteocyte lacuno-canalicular network and its interplay with the cement line in human osteonal bone

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Osteons are bordered by a layer known as the cement line, which functions as an interface separating osteons from each other and from the surrounding interstitial bone (IB). However, the precise sequence of events surrounding the formation of cement lines remains poorly understood. While they provide an appropriate surface for osteoid deposition, they also fulfill a crucial function in bone quality. Cement line appears to contribute to bone strength and affects the connections between osteons, thereby influencing bone mechanosensitivity. In the osteon, there are osteocytes, trapped in lacunae, and connected by tiny channels called canaliculi. Together, they form the osteocyte lacuno-canalicular network (OLCN). Indeed, interactions between different structural elements of bone might be influenced by the quantity of canaliculi that can cross cement lines and connect osteocytes from adjacent osteons. However, despite their importance, the properties of cement lines have not been thoroughly studied. Specifically, the OLCN has been studied within osteons, but very rarely, if ever, outside of them. Indeed, only a few studies have analyzed the network at the border of the osteon, but they contradict each other. Some have observed connections, while others reported none. To address this uncertainty, our goal is to evaluate if there are connections between osteons and their surroundings and if these connections are more likely with IB or other osteons.

To conduct this study, we used two microscopy techniques on the same regions on two bone samples from human femurs: confocal laser scanning microscopy (CLSM) to measure the OLCN in samples stained by rhodamine and quantitative backscattered electrons imaging (qBEI) to measure the local mineral content of the osteon. We also used the open-access tool for image network analysis (TINA) on four different osteons. To conduct this analysis, we had to create a mask and, to choose some parameters to ensure the good analysis of the osteon and an appropriate subvolume to capture the heterogeneities within the network. The analysis started with the evaluation of canalicular density, followed by a qualitative analysis of their orientation. Then, the cells were studied by evaluating their shape according to the mineral content of the osteons and their position within the osteon. Finally, a qualitative analysis was performed on the density of the cells. We observed few connections between the osteon and its surroundings, and these connections are more likely with other osteons than with IB. These sparse connections could be explained by the fact that osteons could behave independently and may not need to communicate and to exchange information We also noticed a very striking absence of network in certain regions of IB which corresponds to the highest mineralized regions. This absence may be due to poor penetration of the rhodamine, potentially hiding the presence of a network. A potential explanation could be that the network may be disrupted with aging and the rhodamine might not be able to penetrate in this specific region. To validate this hypothesis, Focused Ion Beam in dual beam configuration with Scanning Electron Microscope (FIB-SEM) tests should be conducted.

One other interesting finding is that the number of lacunae seems to increase near the cement line. This potential increase in lacuna density could signify a higher activity of osteoblasts at the onset of bone remodeling. Another potential explanation is that the cells can better detect microdamage at the periphery enabling better communication with the external environment. On the other hand, the density of the canaliculi decreases near the cement line and their orientation shifts from being perpendicular to the Haversian canal to parallel as they reach the border. This work offers detailed characterization of the OLCN network within and beyond the periphery of an osteon, revealing sparse connections between osteons and the surrounding bone. These connections likely play a crucial role in bone mechanosensitivity and cellular communication. To further this analysis, increasing the number of samples would allow for the quantification of these connections through the cement lines in relation to tissue age.

Keywords: Cement line, osteocyte network, canalicular density, confocal microscopy, network analysis, canalicular orientation, osteonal bone.