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Human femoral lacuno-canalicular network by X-ray nano-CT

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1. Introduction

Bone mechanobiology is involved in bone biomechanical integrity. Understanding how this mechanobiological remodeling operates *in vivo* is interesting in terms of potential future bone regenerative strategies development. But while both osteoclasts and osteoblasts, bone resorbing and forming cells, are mechanosensitive, the whole bone mechanobiological remodeling is regulated by the osteocytes. These cells are able to identify a damaged region before activating recruited osteoclasts and osteoblasts to resorb this region and form a new bone.

When dealing with bone mechanobiology, it is thus determinant to consider how the osteocytes are mechanically stimulated and how the signalling biomolecules are transported towards the osteoclasts and osteoblasts.

Within bone, osteocytes are trapped in micrometric lacunae all interconnected through submicrometric canaliculi crossing the whole matrix. This Lacuno-Canalicular Network (LCN) is filled with a physiological fluid transmitting the mechanical signals to the osteocytes, and acting as the carrying media for biomolecules. The LCN permeability, involved in both cells mechanosensation and molecular transport, is known to depend on its morphology (Lemaire 2012). But due to the submicrometric canaliculi dimensions and the associated difficulty to characterize it, numerical models used to estimate bone LCN fluids flow are only based on simplified LCN morphologies. In the present study, synchrotron radiation nano-computed tomography (SR- μ CT) has been used to develop a human LCN morphology database to be used in future for more realistic biomechanical models.

2. Materials and methods

The femoral diaphyses from 27 human donors (15 females and 12 males) aged from 50 to 95 years old were harvested. Parallelepiped cortical bone samples ($0.4 \times 0.4 \times 4 \text{ mm}^3$) were cut with the long axis along the haversian canal axis.

The 27 bone samples were imaged at the ESRF (Grenoble, France) on the beamline ID16B. Each sample was scanned at 4 different distances from the source, with 3400 projections recorded at each distance over 360° . The X-ray beam energy was set to 29.6 keV. Phase retrieval was performed using a multi distance Paganin method (Yu 2018). Finally, applying a standard filtered back projection algorithm provided volumes made of $2560 \times 2560 \times 2160$ voxels with a resulting isotropic voxel size of 100 nm.

Osteocytes lacunae were segmented by applying a hysteresis thresholding (Dong 2014). Canaliculi segmentation was performed using a more complex procedure, described in Yu (2018), including the enhancement of their visibility through a Sato's vesselness enhancement method before applying the hysteresis thresholding.

Finally, morphometric parameters, such as the canalicular and LCN (canaliculi + lacunae) total volume fraction (Ca.V/BV and LCN.V/BV in %, respectively) where BV is the bone volume, the canalicular diameter (Ca.D in μm), and the canalicular number in function of the distance to their closest lacuna (Ca.N) were quantified. This parameter was obtained by measuring the number of holes in a surface growing from the lacunar wall to a surface $12 \mu\text{m}$ away from it (Yu 2021).

3. Results and discussion

The proposed image processing protocol was applied to the 27 human femoral volumes corresponding to a physical field of view of $256 \times 256 \times 216 \mu\text{m}^3$. The segmented lacunae and canaliculi are illustrated in Figure 1.

Canalicular volume accounted in average for 0.4% of the total bone volume. This value increased to 1.1 when considering the whole LCN. This means that

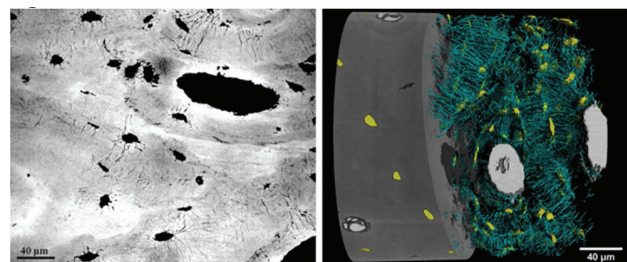
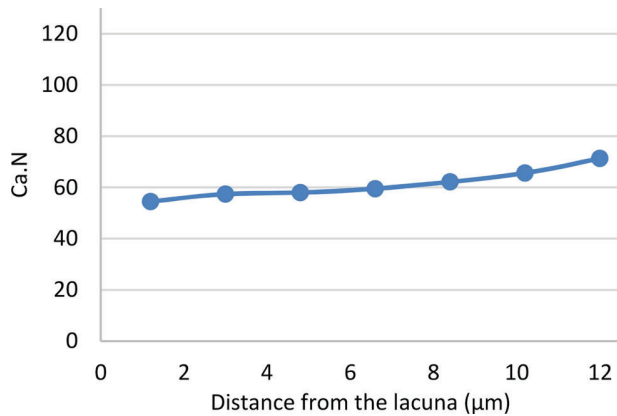


Figure 1. Slice from the VOI and 3D segmented volume with canaliculi and lacunae in blue and yellow.

Table 1. Averaged value and standard deviation (SD) for the LCN morphometric parameters.

	Ca.V/BV (%)	LCN.V/BV (%)	Ca.D (μm)
Averaged	0.4	1.1	0.36
SD	0.1	0.2	0.03

**Figure 2.** Distribution of the number of canaliculi with the distance to the lacunar wall.

the canaliculi volume represented 36% of the LCN, the remaining being occupied by the lacunae. The canalicular diameter was measured at $0.36\ \mu\text{m}$, with quite a low standard deviation (8% of the averaged value) over the investigated population (Table 1).

It was observed that the number of canaliculi increased slightly with the distance to the lacuna, from 54 canaliculi at the lacunar wall to 71 at $12\ \mu\text{m}$ from it (Figure 2). This result suggests that the canalicular network branches out as it spreads from the lacuna. It is interesting to notice the high standard deviation obtained for these measurements.

To accurately predict fluid circulation, LCN morphological properties, such as tortuosity or local thickness, are needed (Lemaire 2012). Similarly, it is known that the canalicular ramification may play a role in maintaining the LCN permeability in the case of canaliculi interruption (Bortel 2022). The current results have shown that the number of canaliculi tends to increase with the distance to a lacuna, suggesting a ramification of the network (Figure 2).

Still, an important variation is observed on this parameter, highlighting the diversified investigated population. For example, the female population presented a trend toward a higher number of canaliculi per lacuna compared to the male population. Additionally, it was observed a high intra-individual variability, with an averaged standard deviation of 31 canaliculi per lacuna for an averaged number of 54 canaliculi per lacuna at $1\ \mu\text{m}$ from it over the investigated population. This result suggests that the LCN morphology varies within

bone matrix. This is in accordance with previous results showing lacunar parameters depending on their localization with bone microstructure (Gauthier 2019).

While in the current study, only global parameters are provided, the data set allows to locally investigate the LCN morphology. Such a local investigation is determinant to better understand the LCN permeability distribution depending on bone organization.

4. Conclusions

To the authors' knowledge, this database is unique and promising in terms of better understanding of the LCN and the further numerical modeling of human cortical bone load-induced fluid flow.

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