QUANTIFICATION OF BONE CELL CONNECTIONS FROM 3D NANO-CT IMAGES

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Introduction: The understanding of bone fragility involved in diseases such as osteoporosis is an active topic of research. At the cellular level, the crucial role of the osteocyte system in bone adaptation was recently highlighted [1]. Osteocytes are the most numerous bone cells embedded in the mineralized matrix. Their bodies are located in spaces called lacunae and they are interconnected through processes hosted in canaliculi. The assessment of the lacuno-canalicular network (LCN) is challenging: i) the LCN has mainly been imaged from 2D microscopic techniques and rarely from 3D techniques; ii) all quantification has been so far performed manually. In previous works, we proposed a unique imaging technique based on Synchrotron Radiation (SR) Computerized Tomography (CT) to image the LCN (isotropic voxel size: 280nm) [2]. Since this method can provide 3D images of LCN enclosing up to thousands of cells, automated quantification methods are needed.

Methods: Herein, we present an automatic 3D quantification method for calculating the number of canaliculi radiating from each osteocyte lacuna (Lc.NCa), which is important for bone permeability [3]. After segmentation of the LCN, based on a line-enhancement filter, the lacunae and canaliculi are discriminated by means of morphological operations and connected component analysis. Then, each lacuna is processed automatically. The number of canaliculi per lacuna, denoted Lc.NCa, is estimated by calculating topological parameters on a specific volume of interest. The mean, standard deviation and distribution of Lc.NCa at different distances from the lacuna surface are obtained for the whole population of lacunae in the image.

Results: The method was validated on a simple phantom and one isolated lacuna by comparison to manual counting. For the isolated lacuna, 22 and 32 canaliculi were calculated at two fixed distances. Then, the proposed method was successfully applied to a 3D SR-nanoCT image of a human femoral cortical bone. Statistical results on 167 lacunae are reported, showing a mean of 41.6 canaliculi per lacuna.

Conclusions: This is the first report of the number of canaliculi calculated directly from a 3D image, enclosing a large population of cells. The result is consistent with previous estimations extrapolated from 2D manual measurements [3]. The different numbers of canaliculi calculated on one isolated lacuna at different distances put in evidence the branching of canaliculi. Although the results rely on the quality of the acquired image and its segmentation, this method is expected to provide new data on the LCN with important impact on the understanding of bone strength. In addition, our method could also be applied to other similar 3D stellate biological objects from 3D images acquired in various modalities.

Acknowledgement/References: [1] L. Bonewald, "The amazing osteocyte" J Bone Min Rese, 26(2), 229-38, 2011.[2] A. Pacureanu, M. Langer, E. Boller, P. Tafforeau, F. Peyrin, Nanoscale imaging of the bone cell network with synchrotron X-ray tomography: optimization of acquisition setup, Med Phys, submitted 2011.[3] T. Beno, Y. Yoon, S. Cowin, and S. Fritton, "Estimation of bone permeability using accurate microstructural measurements," J Biomech, 39(13), 2378-87, 2006.