Journal of Molecular and Genetic Medicine

Minamizaki et al., J Mol Genet Med 2016, 10:4
DOI: 10.4172/1747-0862.1000231

Commentary OMICS International

Nano-Architecture of Osteocyte Lacunae

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Introduction

During bone formation, 20 percent of bone-forming osteoblasts can be trapped in the newly formed bone matrix and become osteocytes. Osteocytes are most commonly found in bone, while their differentiation processes are largely unknown. Osteocytes reside inside spaces in the bone matrix "lacunae," and extend their cell processes through tiny canals called canaliculi, resulting in the formation of an extensive cellular network throughout the bone matrix [1]. Although osteocytes appear to be morphologically inert, recent findings indicate that the cells act as master regulators of bone and mineral homeostasis through the mechanisms underlying the mechanosensing system [2,3]. For example, sclerostin and FGF23 are known to be exclusively produced by osteocytes [4]. The former acts an inhibitor of the Wnt pathway and the resulting decrease in bone formation, and the latter regulates renal phosphate transport to maintain serum phosphate levels that impact bone mineralization. It is also well known that RANKL derived from osteocytes stimulates osteoclast formation and activate bone resorption [4].

Cross-sectional imaging including X-ray computed tomography (CT) and its 3D geometries and properties are used in the clinical routine with a maximum spatial resolution from hundred to dozen μm. The nano-architectures exceeding this resolution currently cannot be visualized [5]. Nano-CT provides high-resolution cross-sectional imaging and significantly exceeds the resolution capacity of established CT systems. Nano-CT technology can achieve a superior spatial resolution at hundred or less nm levels, and hence is anticipated to be utilized for analysis of the nano-architecture in various fields including medical sciences. In general, osteocytes and their lacunae are less than 30 μm in size (long axis length), and accordingly little attention had been paid to the contribution of conventional CT systems to assessing osteocyte properties.

Lacunar volume	(mm^3)
Lacunar volume/bone volume	(%)
Osteocyte lacunar surface	(mm^2)
Lacunar surface/lacunar volume ratio	(1/mm)
Lacunar surface/bone volume	(1/mm)
Lacunar surface convexity index	(1/mm)
Lacunar diameter	(mm)
Lacunar linear density	(1/mm)
Lacunar separation	(mm)
Degree of anisotropy (non-random alignment of osteocyte lacunae)	Scalar value between zero and the degree
Fractal dimension (complexity of osteocyte lacunar surface)	D=ratio of the numbers
Lacunae number	n
Lacunar number/bone volume	($1/mm^3$)
Average lacunar length	(mm)
SD lacunar thickness	(mm)
SD lacunar separation	(mm)
Lacunar diameter distribution	(mm)
Lacunar separation distribution	(mm)
Eigenvalue (vertical direction, lateral direction, longitudinal direction)	Special set of scalars
(Provided by Brukar micro CT, Kontich, Belgium).	

Table 1: Osteocyte lacunar parameters.

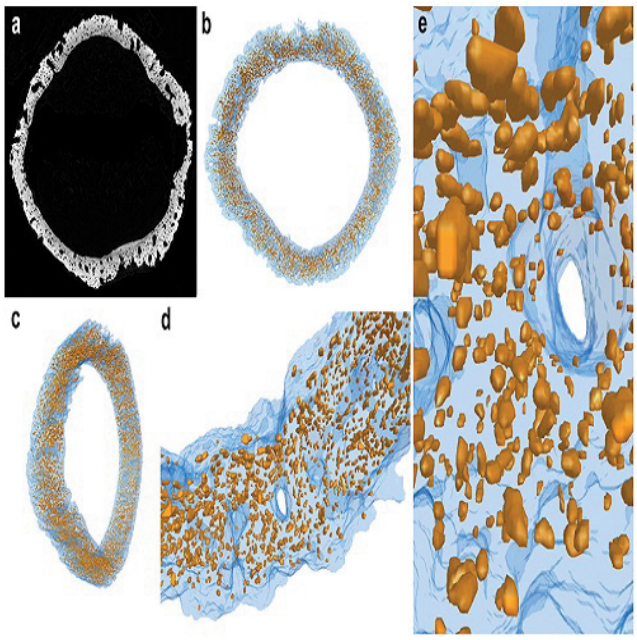

Figure 1: Nano-CT images of osteocyte lacunae in the mouse femur. The femur from the 6-week-old *kl*/+ male mouse was used for analysis. Nano-CT scan was performed using a high-resolution nano-CT system (voxel size, 600 nm; Sky Scan 2211, SkyScan NV, Kontich, Belgium). The energy of the beam was set to 45 kV, and an aluminum filter (0.5 mm) was used. After standardized reconstruction by using NRecon software (Brukar micro CT, Kontich, Belgium), 3D modeling of the samples was carried out by using CTvox software (Brukar) and the data sets for each bone section were analyzed using CTan software (Brukar). Nano-CT analysis was performed on 401 slices of 600 nm thickness in the femoral diaphysis. (a) A reconstructed slice of the overview scan. (b-e) 3D images of osteocyte lacunae. Two different view directions at low magnification (b and c), and higher magnification views of b (d and e). Light blue, cortical bone; Brown, osteocyte lacunae.

Osteocytes change activities or die, accompanied by their morphologies, throughout life as well as in pathological situations [2-4]. Functional and morphological anomalies of osteocytes are reflected in osteocyte lacunar density, volume, shape, and anisotropy which was evaluated by Syncrotron Radiation micro-CT and nano-CT [6-8].

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Received November 03, 2016; **Accepted** November 17, 2016; **Published** November 21, 2016

Citation: Minamizaki T, Ahmed F, Yoshiko Y (2016) Nano-Architecture of Osteocyte Lacunae. J Mol Genet Med 10: 231 doi:10.4172/1747-0862.1000231

Thus, criteria for osteocyte lacunae may be beneficial for the diagnosis and management of bone development and health. Currently, multiple parameters are available to define the nano-architectures of osteocyte lacunae by nano-CT (Table 1).

Three-dimensional rendering of nano-CT images shows multiple types of osteocyte lacunae in the mouse cortical bone (Figure 1), suggesting the remarkable ability of nano-CT. The total number of osteocytes in the adult skeleton is reported to be ~ 42 billion. As for osteocyte dendritic projections (cell networks), the total number reaches 100 times more than that of osteocytes [1]. These data allow us to better understand osteocyte properties by evaluating as many as possible of osteocyte lacunae in the site of interest. In terms of covering a broad range of osteocyte lacunae with high resolution, nano-CT exhibits its high performance.

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