Discovery of new bone calcium dissolution phenomenon: images of calcium exuding from bone captured for the first time using a high-sensitivity 3D X-ray microscope at SPring-8

Osteocytes extend the canaliculi in all directions and interconnect with each other. Osteocytes and bone canaliculi networks fill the inside of bones [1]. The bone remodeling process is thought to occur as follows. When a fracture occurs in a bone, osteocytes detect the fracture using canaliculi and then transmit signals to osteoblasts on the bone surface via the bone canaliculi to promote the differentiation of osteoclasts, which resorb bone and break down the damaged section. Osteoblasts are then formed to repair the bone resorption lacunae. It was previously thought that the main purpose of bone canaliculi networks was to transmit signals concerning bone repair and to supply nutrients.

The serum calcium concentration is maintained at a constant level (serum calcium homeostasis). Too much or too little serum calcium can pose vital risks. When a requirement for calcium arises in the body because of pregnancy or disease, the bone surface is broken down by osteoclasts that resorb bone and dissolve calcium. Only osteoclasts were previously thought to maintain the serum calcium concentration through bone resorption. However, the fact that an artificially created mouse model without any osteoclasts was able to survive suggests an alternative mechanism for homeostatic calcium maintenance. The process of supplying bone calcium through osteoclast activation has the disadvantage of weakening bone through repeated bone resorption because resorption takes a week but bone remodeling takes several months. Therefore, we investigated whether the serum calcium concentration can be maintained using osteocytes.

The tibial cortical bone of 4–14-week-old female mice was observed using a newly conceived Talbot-defocus multiscan X-ray microscope [2], which was constructed at the undulator beamline **BL20XU**, SPring-8. Because the beam radiated from SPring-8 is a strong parallel X-ray, it is possible to create a monochrome X-ray microscope. We assembled a 6 m microscope in order to achieve ×20 magnification with a pixel size of 0.2 μm. The specimen was rotated while the images were taken, and three-dimensional (3D) computed tomography (CT) revealed hundreds of osteocytes and bone canaliculi networks. The same site was observed using two different methods, and two types of highly sensitive, high-resolution 3D images over a wide field of view were produced (Fig. 1). Using Talbot phase contrast imaging [3], it was possible to differentiate the brightness of two points 1 μm apart. Using defocus phase contrast imaging [4], it was possible to produce clear 3D images of bone canaliculi of 0.2 μm diameter emerging from the osteocytes. It was also possible to differentiate the calcium concentration in the area surrounding the osteocytes and bone canaliculi networks by observing the same spot using the two different methods.

(1–1) We captured an image showing a phenomenon wherein bone calcium was depleted in a concentric manner over an area of approximately 4 μm width that surrounded the bone canaliculi extending from the osteocytes (Fig. 1(b) and Fig. 2). An image showing the phase parallel to the bone canaliculi revealed decreased calcium concentration in accordance with the canaliculi path (Fig. 1(b,d)).

(1–2) The area of bone was divided into an osteocyte/bone canaliculi zone with a decrease in calcium concentration surrounding the canaliculi (Fig. 1(a) zone L)), a zone with no change in calcium concentration (Fig. 1(a)-H)), and a zone midway between the two (Fig. 1(a)-M)).

(1–3) A cross-sectional image of the bone canaliculi showed a greater extent of bone loss near the center in almost all of the canaliculi, (red spots in Fig. 2).

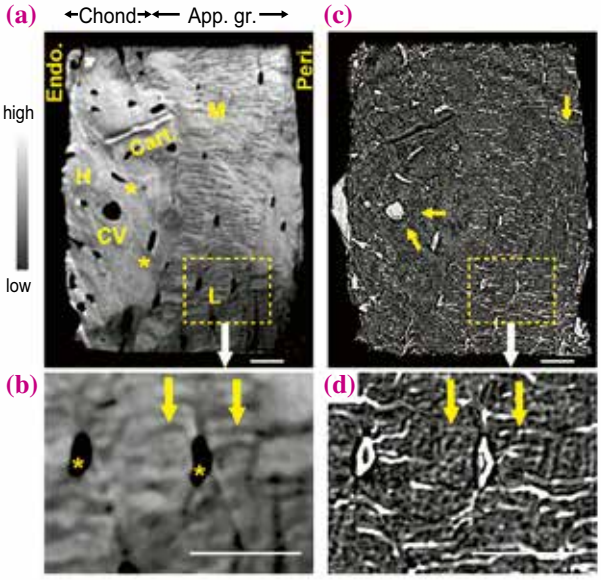

Fig. 1. Synchrotron X-ray tomographic images of tibial cortex in 4-week-old mice. **(a)** Degree of mineralization obtained by Talbot phase-contrast imaging. CV, capillary vessel. Asterisks, osteocytes. **(c)** Osteocytic lacunae and canaliculi observed by defocus phase-contrast imaging. Arrows, osteocyte canaliculi.**(b)** and **(d)** Enlargements of the rectangular areas in (a) and (c), respectively. Scale bars, 20 μm

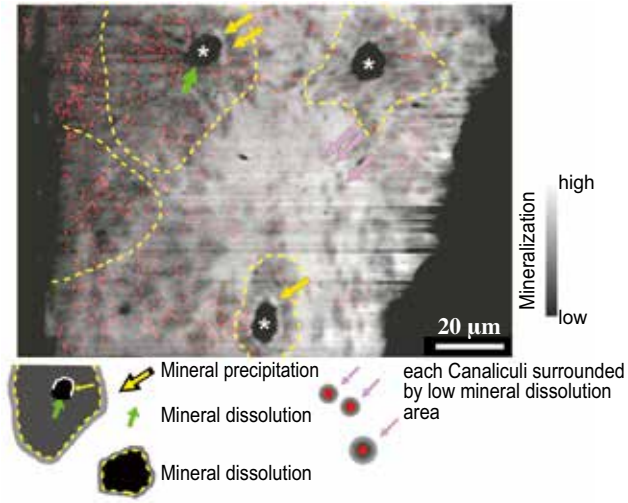

Fig. 2. Coronal section perpendicular to canaliculi. An overlaid image of the degree of mineralization, visualized by Talbot phase-contrast imaging (grayscale), and the canaliculi position, visualized by defocus phase-contrast imaging (red). Yellow dotted lines, low mineralization zone around osteocytes. Asterisks, osteocytes. Yellow arrows, perilacunar hypermineralization.

Osteocytes surrounded by a narrow area of calcium dissolution have high perilacunar mineralization at the edge (yellow arrows in Fig. 2). In contrast, osteocytes on the side with a decreased calcium concentration exhibited low perilacunar mineralization at the edge (green arrow in Fig. 2). It is thought that the H, M, and L zones in Fig. 1 reflect the progress of calcium dissolution.

Bone canaliculi were closely connected to capillary vessels and the bone surface (yellow arrows in Fig. 1(c)). It appears that serum calcium is supplied and deposited into bones via the bone canaliculi and that calcium is constantly being accumulated in bones by this mechanism. This suggests that the L zone was created as a result of bone dissolution by osteocytes after which calcium is replenished in the bone over a long period of time and that the accumulation and recovery of lost calcium occur. Therefore, it is conceivable that the high perilacunar mineralization in the H zone occurs when the calcium concentration increases (Fig. 3).

A study of different mouse bone specimens under different conditions demonstrated the following characteristics with regard to the bone calcium dissolution phenomenon next to the bone canaliculi [5]:
1 - The phenomenon was observed regardless of the age of the mice.
2 - The phenomenon was observed with Fos KO mice without osteoclasts. Osteocytes appear to dissolve calcium around bone canaliculi independent of osteoclasts.
3 - Calcium dissolution even occurred in many lactating mouse specimens.

On the basis of the above findings, it is conceivable that osteocytes and bone canaliculi networks are involved in bone mineral dissolution and storage and that this action is not accompanied by structural changes in bone tissue. Many drugs used in the treatment of osteopenia suppress bone resorption by osteoclasts, causing old bone to be left over, and the bones become frail. It is hoped that new methods of treatment without side effects can be developed by controlling bone dissolution, the formation of new bone, and the action of osteocytes without bone dissolution.

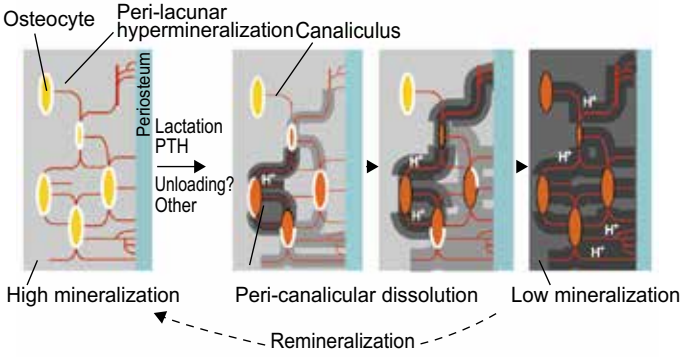

Fig. 3. Model of pericanalicular demineralization. Pericanalicular dissolution starts with a subset of osteocytes in high mineralization zones. Perilacunar hypermineralization is partially or totally lost as pericanalicular demineralization progresses, giving rise to larger zones of intermediate to low mineralization. Yellow ovals: osteocytes that do not direct demineralization. Red ovals: osteocytes directing pericanalicular demineralization. High mineralization zones are likely restored by remineralization.

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