Fine Structure of Porous Canal Network in Cortical Bone

Blood flow is axiomatically the basis of bone growth, remodeling, and repair because it supplies oxygen, minerals, humoral regulatory factors, bone cell precursors, and other materials requisite for bone integrity [1,2]. In cortical bone, microvessels run through a network of porous canals. Therefore, we can estimate the state of cortical bone perfusion on the basis of the state of the canal network structure. However, despite its importance in bone biology, there have been only a few studies on the canal network structure owing to methodological difficulties. Recently, X-ray CT based on synchrotron radiation (SRCT) has created new opportunities for the 3D analysis of cortical bone microstructures [3,4]. Using monochromatic X-rays with an extremely high intensity enables the removal of beam-hardening artifacts and quantitative imaging with a high signal-to-noise ratio [5], which are beneficial in determining the metric properties of cortical canals such as diameters and volumes.

Figure 1 shows a 2D image of a cortical transverse section in a tibial segment harvested from a 14-week-old male Wistar rat (left) and the section's linear absorption coefficient distribution (right). The image was obtained using 20-keV X-rays and the CT system at beamline **BL20B2**. Reconstruction was carried out using a 2D filtered backward projection algorithm based on radiographic images acquired over an angular range of 0°-180° with 0.5° steps, providing contiguous images composed of 1000 × 1000, 5.83-μm pixels. The use of monochromatic X-rays accurately provided the differentiated peak of the linear absorption coefficient corresponding to pure bone, allowing simple thresholding for the bone segmentation. Laboratory CT lacks this advantage because it uses polychromatic X-rays.

The threshold value was determined to be 5.3 cm^{-1} by comparing binarized SRCT images and the light micrographs of the nondecalcified sliced sample showing the same transverse section. Assuming that the bone X-ray absorption is described as a two-phase mixture (hydroxyapatite and light elements), a linear relationship holds between the linear absorption coefficient (μ_{bone}) and hydroxyapatite density (ρ_{HAp}). To confirm this relationship, a dipotassium hydrogen phosphate (K_2HPO_4) water solution was used as a substitute for hydroxyapatite. The linear regression of the linear absorption coefficient and the concentration of the K_2HPO_4 water solution obtained using 20-keV X-rays provided $\mu_{bone} = 5.45 \cdot \rho_{HAp} + 0.81$ ($r^2 > 0.999$), showing that a threshold value of 5.3 cm^{-1} is equivalent to a hydroxyapatite density of 0.82 g/cm^3.

Disuse or immobilization induces bone atrophy. However, its effect on the canal network structure in cortical bone is poorly understood. In Fig. 2, the volume-rendered 3D display of a pair of tibial diaphyses and close-up views of the boxed regions are shown. These tibiae were harvested from a 14-week-old rat treated by unilateral sciatic neurectomy of the left hindlimbs. Neurectomy-induced disuse leads to canal network regression as well as bone atrophy. The indexes characterizing the canal

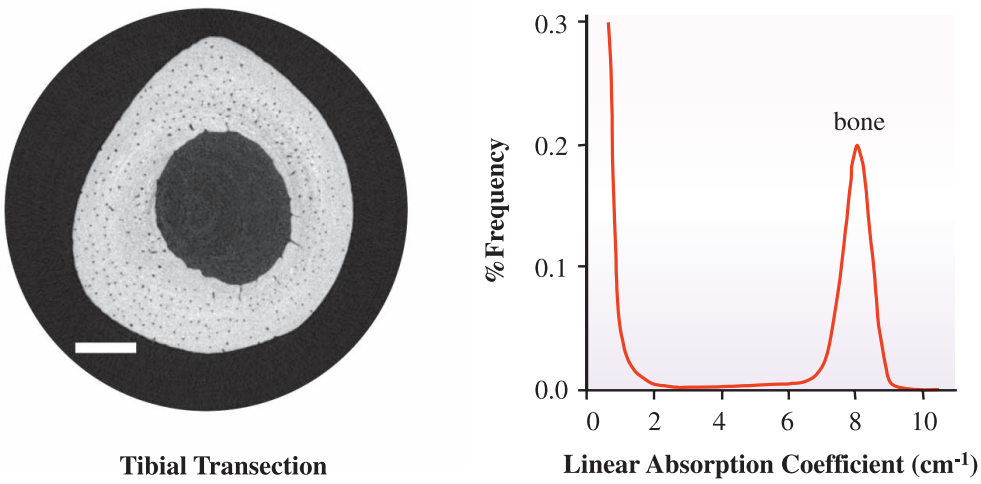

Fig. 1. Reconstructed 2D image of tibial cortical transverse section of rat hindlimb (left) and such section's relative linear absorption coefficient distribution (right). Bar: 200 μm.

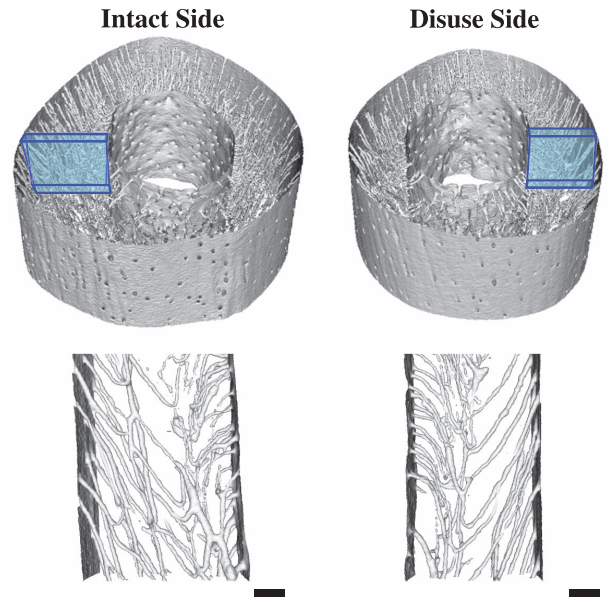

Fig. 2. Image of pair of tibial diaphyses harvested from a rat subjected to unilateral sciatic neurectomy, generated by 3D isosurface rendering of reconstructed 2D images. The boxed regions are enlarged below. Bar: 150 μm. The bone mineral density on the isosurface is 0.82 g/cm^3.

network structure are shown in Fig. 3, indicating that disuse bone atrophy is accompanied by cortical canals, which are decreased in cross-section, sparsely distributed and connected in a tree-like manner. Considering that canals possibly contain capillary vessels structure, this canal network regression could be translated as the regression of the vascular network, leading to a lower perfusion rate, higher flow heterogeneity, and a smaller surface area for oxygen and nutrients needed by cortical bone cells.

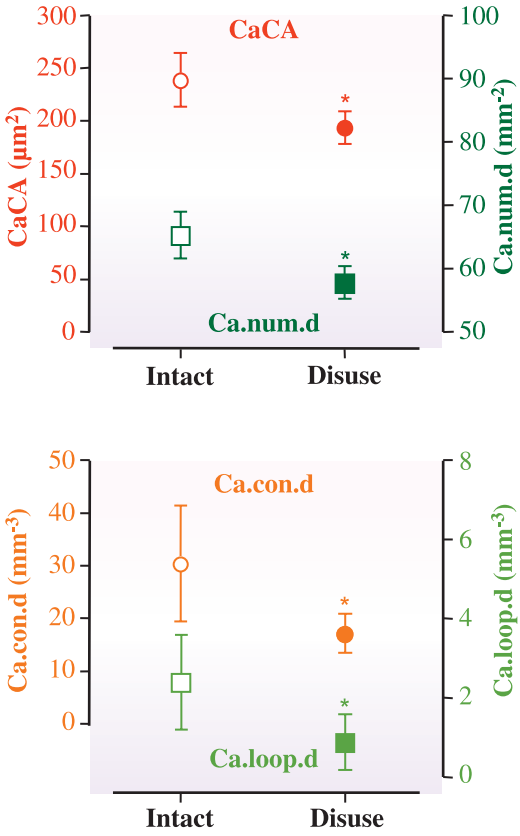

Fig. 3. Plots (mean±SD, n=8) of canal cross-sectional area (CaCA, μm^2), density of canals running longitudinally (Ca.num.d, mm^{-2}), density of canal connections (Ca.con.d, mm^{-3}), and density of canal loops (Ca.loop.d, mm^{-3}). *P<0.05 *vs* intact bone (Wilcoxon matched-pairs signed-rank test).

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References

[1] M. Brookes, W.J. Revell: Blood Supply of Bone: Scientific Aspects, Springer-Verlag, (1998), London, UK.
[2] M.L. Brandi and P. Collin-Osdoby: J. Bone Miner. Res. **21** (2006)183.
[3] F. Peyrin *et al.*: Cell Mol. Biol. **46** (2000)1089.
[4] *T. Matsumoto, M. Yoshino, T. Asano, K. Uesugi, M. Todoh and M. Tanaka: J. Appl. Physiol. **100** (2006) 274.*
[5] U. Bonse and F. Busch: Prog. Biophys. Mol. Biol. **65** (1996)133.