



# Haversian system of compact bone and comparison between endosteal and periosteal sides using three-dimensional reconstruction in rat

Jeong-Nam Kim<sup>1</sup>, Jun-Young Lee<sup>1</sup>, Kang-Jae Shin<sup>2</sup>, Young-Chul Gil<sup>2</sup>, Ki-Seok Koh<sup>2</sup>, Wu-Chul Song<sup>2</sup>

<sup>1</sup>Department of Biomedical Laboratory Science, Masan University, Masan, <sup>2</sup>Department of Anatomy, Research Institute of Medical Science, Konkuk University School of Medicine, Seoul, Korea

**Abstract:** The current model of compact bone is that of a system of Haversian (longitudinal) canals connected by Volkmann's (transverse) canals. Models based on either histology or microcomputed tomography do not accurately represent the morphologic detail and microstructure of this system, especially that of the canal networks and their spatial relationships. The aim of the present study was to demonstrate the morphologic pattern and network of the Haversian system and to compare endosteal and periosteal sides in rats using three-dimensional (3D) reconstruction. Ten Sprague-Dawley rats aged 8–10 weeks were used. The femurs were harvested from each rat and fixed, decalcified with 10% EDTA-2Na, serially sectioned at a thickness of 5  $\mu\text{m}$ , and then stained with hematoxylin and eosin. The serial sections were reconstructed three-dimensionally using Reconstruct software. The Haversian canals in the endosteal region were found to be large, highly interconnected, irregular, and close to neighboring canals. In contrast, the canals in the periosteal region were straight and small. This combined application of 3D reconstruction and histology examinations to the Haversian system has confirmed its microstructure, showing a branched network pattern on the endosteal side but not on the periosteal side.

**Key words:** Haversian system, Compact bone, Three-dimensional reconstruction, Microstructure, Histology, Rat

Received January 9, 2015; Revised September 11, 2015; Accepted October 1, 2015

## Introduction

The morphological and functional unit of the bone is the Haversian system, with textbooks diagrams showing Haversian or central (longitudinal) canals connected by Volkmann's or perforating (transverse) canals. Officially, Haversian and Volkmann's canals are "nutrient and perforating canal" in

Terminologia Histologica [1-3]. The canals have a concentric lamellar organization and are of equal size. The bone is vascularized by vessels that penetrate the matrix from the periosteum.

The microstructure of the compact bone is important to bone remodeling and modification in pathologic conditions. Therefore, knowledge of the microstructure of compact bone such as the Haversian system is important to understanding remodeling activity, distribution analysis, the pathogenesis of the osteoporosis, and other mechanisms. However, many studies of the Haversian system have focused only on the canals themselves due to methodology limitations associated with cross-sectional histology and microcomputed tomography [4-7], rather than on the general canal system

### Corresponding author:

Wu-Chul Song

Department of Anatomy, Konkuk University School of Medicine, 120 Neungdong-ro, Gwangjin-gu, Seoul 05029, Korea

Tel: +82-2-2030-7819, Fax: +82-2-2030-7845, E-mail: anatomy@kku.ac.kr

Copyright © 2015. Anatomy & Cell Biology

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

or the spatial relationships between the two types of canals. Knowledge of the overall composition of the Haversian system as well as the constituent canals themselves is very important when investigating changes relative to the normal system.

The aim of the present study was to demonstrate the actual microstructure and morphologic pattern of the Haversian system of compact bone using histology and three-dimensional (3D) reconstruction, rather than relying on virtual images. This described reconstruction technique can provide a general view of the architecture of the Haversian system and further the understanding of pathologic progression of the compact bone.

## Materials and Methods

### Materials and sampling

Twenty Sprague-Dawley rats aged 8–10 weeks were used. The animals were perfused with 150 ml of saline followed by 600 ml of fixative containing 4% paraformaldehyde in phosphate-buffered saline. The femur was harvested and decalcified in 5% EDTA-2Na. The specimens were trimmed to 3 mm to make them easier to handle and to facilitate the acquisition of images during fixation. Each specimen was obtained from the anterior midshaft of the femur.

### Tissue processing

After dehydration and infiltration, the paraffin-embedded specimens were cut transversely into 150 to 200 serial sections at a thickness of 5  $\mu\text{m}$  for 3D reconstruction. The sectioned tissues were then stained with hematoxylin and eosin.

### 3D reconstruction

All of the stained sections were photographed using a

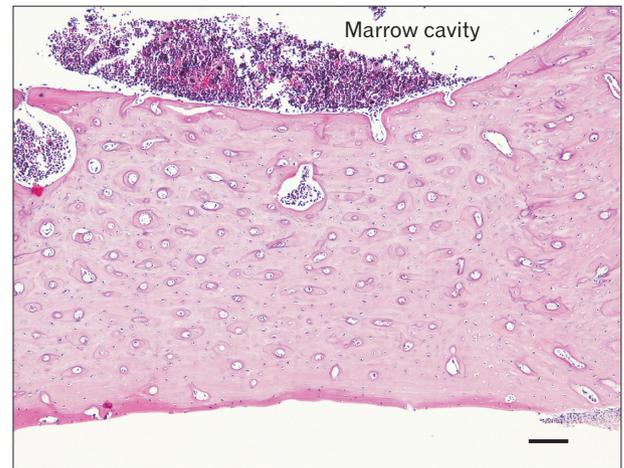


Fig. 1. Transverse section of the rat femur stained with hematoxylin and eosin. Scale bar=100  $\mu\text{m}$ .

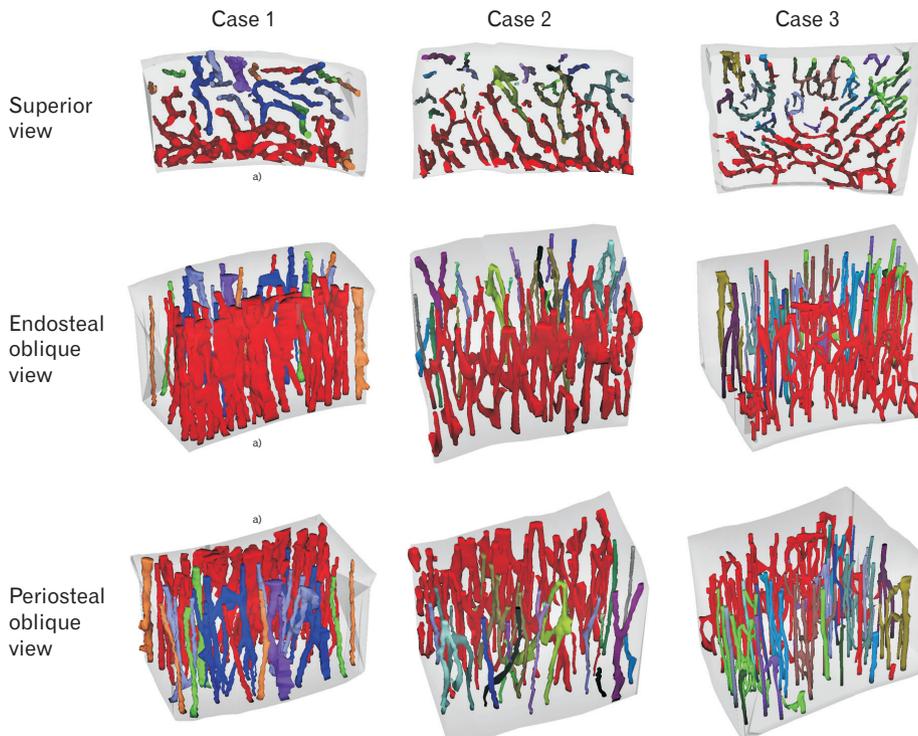


Fig. 2. Three-dimensional reconstruction of the Haversian system. Different colors represent the different networks of the Haversian and Volkmann's canals. Transparent gray shows the outline of the bone fragment. Many, large, and complex reddish networks of the Haversian and Volkmann's canals are located on the endosteal side, while relatively simple networks are located on the periosteal side. All images have the same magnification. <sup>a)</sup>Endosteal side.

2,048×1,536-pixel digital CCD camera (DP70, Olympus, Tokyo, Japan). The serial images were aligned manually using computer software, the Haversian and Volkmann's canals were segmented manually, and all structures were reconstructed three-dimensionally. "Reconstruct" software was used to produce the 3D images of the Haversian system (this software can be downloaded from <http://synapses.clm.utexas.edu/tools/reconstruct/reconstruct.stm>) [8].

## Results

The Haversian canals were arranged into concentric rings and connected to each other by oblique perforating canals in the transverse sections (Fig. 1). However, the network pattern, spatial relationship between Haversian and Volkmann's canals, and difference between endosteal and periosteal sides were difficult to identify in the transverse histology sections. In contrast, all of these features were clearly evident in the reconstructed 3D models (Fig. 2). The basic structure comprises Haversian canals that are large in the endosteal region and highly interconnected. The canals in the endosteal region appear irregular and close to neighboring canals. In the other hand, the canals of the periosteal region are relatively small and less interconnected; that is, the network density is significantly higher in the endosteal region than in the periosteal region. The Haversian canals are straight and long and parallel to the length of femur. The Volkmann's canals run perpendicular to the Haversian canals, interconnecting the latter with each other and the periosteum, and extending in random directions at various angles.

## Discussion

Haversian canals are a series of tubes around narrow channels formed by lamellae. The Haversian canals surround blood vessels and nerve fibers throughout the bone and communicate with osteocytes. The canals and the surrounding lamellae are called a Haversian system (or an osteon). A Haversian canal generally contains one or two capillaries and nerve fibers. The spaces between Haversian systems contain interstitial lamellae. The osteonal pattern of compact bone is gradually built around the intracortical vessels by the progression of the cutting cones in a process of secondary remodeling; therefore, the central canal size can be used as an index of the remodeling activity [9].

Textbook diagrams of the Haversian and Volkmann's canals

in the Haversian system indicate that the central canal size is equal in the endosteal and periosteal regions of compact bone [1-3]. However, we found that the Haversian system has a complex pattern of organization that is dominated by branching or Volkmann's canals. Our method of combining 3D reconstructions with histology examinations has confirmed the actual morphologic pattern of the Haversian system.

The main result of the present study is that the Haversian system is convoluted and closely interconnected in the endosteal region, while being straight in the periosteal region. The canals are significantly larger in the endosteal region than in the periosteal region. Moreover, there are more interstitial lamellae in the periosteal region.

The microstructure of the Haversian system is characterized by a branched network pattern that differs between the endosteal and periosteal sides. Because previous studies of the pathology of the compact bone during osteoporosis have generally focused on the thickness of the compact bone, the present results are useful in providing a general view of the architecture of the Haversian system and for understanding pathologic progression of the compact bone. In particular, in cases of osteoporosis the thickness of the cortical bone is reduced even though the external diameter remains almost intact. It might be that the degree of bone resorption is much greater in the endosteal region due to the presence of a large and highly interconnected Haversian system there.

The present results have demonstrated that the network of the Haversian system can be revealed only by combining 3D reconstruction with histology observations. We therefore further suggest that 3D reconstruction could also be a highly effective tool in other areas of morphology research.

## Acknowledgements

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund)(313-2008-2-E00005).

## References

1. Kessel RG, Kardon RH. Tissues and organs: a text-atlas of scanning microscopy. New York: W.H. Freeman and Co.; 1979.
2. Ovalle WK, Nahirney PC. Netter's essential histology. Philadelphia: Elsevier; 2008. p.146.
3. Mescher AL. Junqueira's basic histology. 13th ed. New York: McGrawHill; 2013. p.145-8.
4. Tanaka M, Yamashita E, Anwar RB, Yamada K, Ohshima H,

- Nomura S, Ejiri S. Radiological and histologic studies of the mandibular cortex of ovariectomized monkeys. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2011;111:372-80.
5. Particelli F, Mecozzi L, Beraudi A, Montesi M, Baruffaldi F, Viceconti M. A comparison between micro-CT and histology for the evaluation of cortical bone: effect of polymethylmethacrylate embedding on structural parameters. *J Microsc* 2012;245:302-10.
6. Britz HM, Jokihaara J, Leppänen OV, Järvinen T, Cooper DM. 3D visualization and quantification of rat cortical bone porosity using a desktop micro-CT system: a case study in the tibia. *J Microsc* 2010;240:32-7.
7. Cooper DM, Matyas JR, Katzenberg MA, Hallgrímsson B. Comparison of microcomputed tomographic and microradiographic measurements of cortical bone porosity. *Calcif Tissue Int* 2004;74:437-47.
8. Fiala JC. Reconstruct: a free editor for serial section microscopy. *J Microsc* 2005;218(Pt 1):52-61.
9. Pazzaglia UE, Zarattini G, Giacomini D, Rodella L, Menti AM, Feltrin G. Morphometric analysis of the canal system of cortical bone: An experimental study in the rabbit femur carried out with standard histology and micro-CT. *Anat Histol Embryol* 2010;39:17-26.