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Review

Osteocyte bioimaging

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ABSTRACT

Background: Newly developed visualization methods often lead to breakthroughs in the bioscience field. In particular, the ability to reveal temporal–spatial responses in cells, while visualizing molecular events through bioimaging techniques is very important. One such event is the regulation of bone remodeling by osteocytes. It is thought that osteocyte processes sense the flow of interstitial fluid that is driven through the osteocyte canaliculi by mechanical stimuli caused in the bone. However, the precise mechanism by which the flow elicits a cellular response is still unknown.
Highlight: It is critical to obtain precise morphological and/or morphometrical data from osteocytes and their surrounding microenvironment. In this review, we describe our application of confocal laser scanning microscopy to visualize osteocyte morphology in the bone and the combination of ultra-high voltage electron microscopy (UHVEM) and computer simulation of fluid flow to reveal the mechanosensitivity of osteocytes in the bone.
Conclusion: The osteocyte network in the bone as well as the microstructure of osteocyte cell processes and the surrounding bone matrix were visualized. We found fluorescence to be useful for studying the osteocyte network morphology. Additionally, the combination of UHVEM and computer simulation is a powerful tool to study the fluid flow in osteocyte canaliculi.

Contents

1. Introduction

It is well known that osteocytes are the most predominant cells in the bone and are located inside the mineralized bone matrix. Their cytoplasmic processes form a complex intercellular network via gap junctions. In this network, osteocytes are thought to be the principal cells responsible for sensing mechanical stimuli and transporting signals that coordinate the adaptive bone-remodeling response [1]. Overall, it is thought that mechanical loading-induced matrix strain causes a flow of interstitial fluid around the osteocyte processes [2,3]. However, the mechanism by which this fluid flow excites the osteocytes remains unclear. Therefore, it is very important to obtain precise morphological and/or morphometrical data from osteocytes and their surrounding matrix. In the first chapter, we introduce our application of confocal laser scanning microscopy [4] to characterize the osteocyte network in the bone. In the second chapter, we describe a high-resolution analysis of osteocyte morphology via ultra-high voltage electron microscopy (UHVEM) [5]. In the third chapter, we

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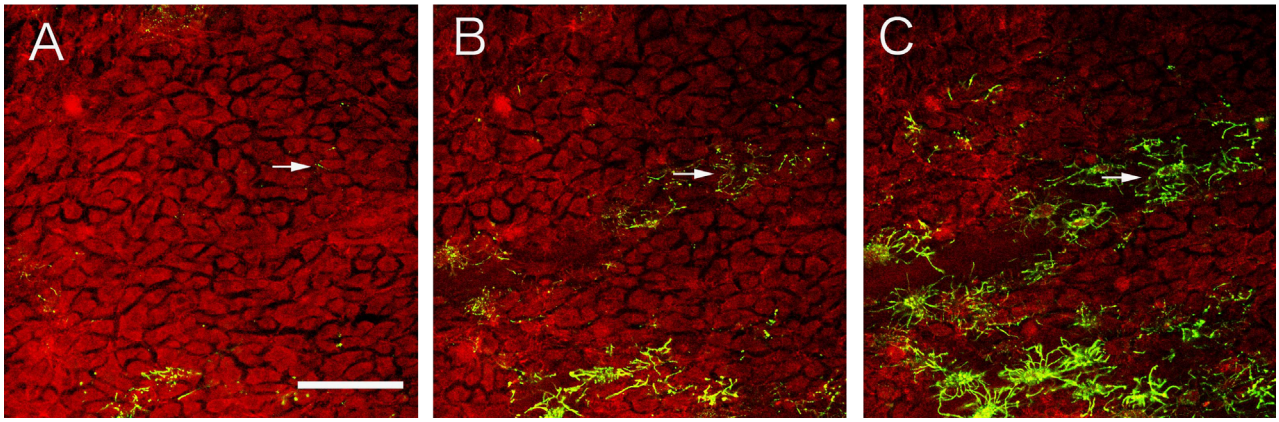

Fig. 1. Serial histotomography by CLS microscopy. The fluorescent images show the cells stained with Texas Red-X phalloidin (red) and OB7.3 (green). (A) The vascular-facing surface of the osteoblast layer. Arrow shows the tip of the osteocyte processes. (B) Two and a half micrometers from the first layer. Arrow shows the osteocyte processes. (C) Five micrometers from the first layer. Arrow shows the osteocyte. Bar = 50 μm. Data are from Kamioka et al. [6], with permission to reprint from Elsevier Science Inc. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

demonstrate how the fluid flow causes fluid shear stress surrounding the osteocyte processes using computer simulation based on a three-dimensional nanoscale model of osteocyte canaliculi.

2. Bioimaging of osteocytes for morphological analysis by fluorescence

To visualize the osteocyte network, we first employed a combination of confocal laser scanning (CLS) microscopy and differential interference contrast (DIC) microscopy for the observation of osteocytes in mineralized embryonic chicken calvariae [6]. The CLS microscopic images identified whole cells stained with Texas-Red-X phalloidin as well as osteocytes immunofluorescently labeled by the monoclonal antibody OB7.3, which specifically identifies chicken osteocytes [7]. The complementation of CLS microscopy with DIC microscopy in the same confocal layer revealed the 3D organization of the cells as well as the lacunar and canalicular walls. In this study, we revealed for the first time that osteocytes elongate their processes to cells on the bone surface and that their dendritic processes run through the osteoblast layer, implying that there is a possibility for a direct contact of osteocytes with the marrow-residing cells (Fig. 1). The distribution of osteocyte processes between the osteocytes and osteoblasts was also analyzed. The ratio of the processes through the osteoblast layer was $2.63 \pm 4.17\%$ [6]. Therefore, there is a possibility that stress-sensing osteocytes propagate the information to surrounding cells in the bone.

3. UHVEM to visualize osteocyte morphology

Although the fluorescent analysis of the osteocyte network is useful for examining a large sample volume, a high-resolution analysis was necessary for the precise histomorphological analysis of the osteocyte cell processes and osteocyte canaliculi. The diameter of the cell processes and osteocyte canaliculi is typically less than 250 nm, which is the theoretical resolution of the fluorescent analysis. Thus, we employed electron tomography to reveal the 3D morphology of the osteocytes at the nanometer level. UHVEM (3 MeV) [5] was utilized to reconstruct the osteocyte network in 3-μm-thick sections. We succeeded in acquiring transmission electron micrographs from 3-μm-thick sections of the bone at a resolution of 8 nm/pixel and reconstructed the three-dimensional morphology of the osteocytes [8] (Fig. 2). We focused on the young osteocytes in the modeling site of chick calvaria. Reconstructed young osteocytes clearly showed that the surfaces of the cell body and processes were not smooth, but irregular.

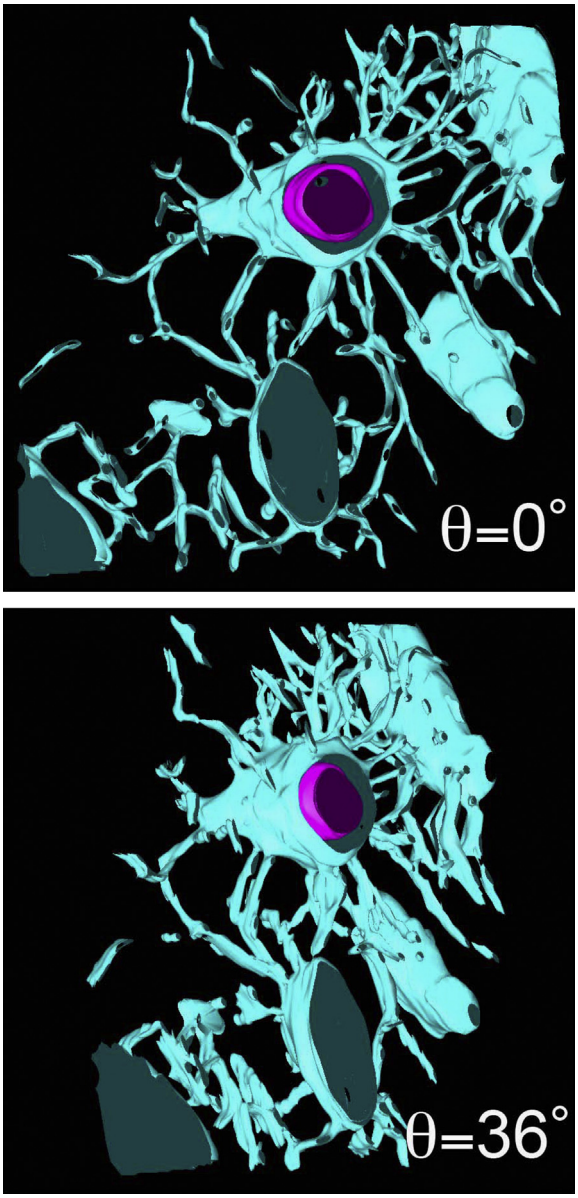

Fig. 2. Three-dimensional reconstruction of young chick osteoid-osteocytes. The reconstructed images were rotated and are shown at 0° and 36° in the horizontal plane. Data are from Kamioka et al. [8].