THE INTRAOSSEOUS FLUID PRESSURE GENERATION IN LACUNOCANALICULAR NETWORK OF TRABECULAE

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ABSTRACT
The intraosseous pressure generation and fluid flow in lacunocanalicular network of trabeculae could be important to the remodeling process of cancellous bone. Due to the technical difficulty, experimental information about the amount of intraosseous pressure generation in lacunocanalicular network of trabeculae is never measured. In this study, the maximum intraosseous pressure generation in micro-trabecular specimens within the elastic range was measured in vitro using a specially designed micro-experimental setup representing the most restrictive fluid flow boundaries around microscopic bovine vertebral trabecular specimens. Then, a quasi-static loading (9 μm/min) was applied up to the strain of 0.4 % with measuring intraosseous pressure generations in the undrained and drained conditions. 49.2 ± 4.45 KPa of intraosseous pressure generation at the 0.4% strain was found in the undrained condition. In contrast, no intraosseous pressure generation was measured in the drained condition. The result could let us know the amount of a possible maximum intraosseous pressure generation in lacunocanalicular network of trabeculae within the elastic range.

KEY WORDS
Lacunocanalicular Network, Trabecular, Intraosseous Pressure, Microscopic Property Measurement, Biomechanics

1. Introduction

Cancellous bone shows a structure composed of an interconnected series of trabeculae. The interconnected series of trabeculae form the inter-trabecular macro pore space that the size of pore is in the order of 100 to 500 μm in diameter. In contrast, trabeculae have the other pore space that is mostly composed of the lacunocanalicular system. The size of the pore (0.5 to 50 μm in diameter) is very much smaller than that in the inter-trabecular pore space. The pore space in trabeculae helps to form the connected cellular network that all bone cells except osteoclasts are extensively connected by the cell processes of osteocytes. Each osteocyte buried in a lacuna has about 80 cytoplasmic processes that lie within canaliculae arrayed three dimensionally. As a result, the pore space in trabeculae is interconnected by the lacunocanalicular system filled with the osteocytes, cytoplasmic processes, bone fluid, and lacuna fluid [1]. In addition, canaliculi open to the extracellular fluid and surface of trabeculae. Thus, intraosseous fluids having a viscosity of physiological saline water in and near trabeculae circulate for the nutrition and metabolism of the osteocytes [2].

It is understood that the micro-anatomical intraosseous fluids in trabeculae circulate by external loading of a bone organ causing intraosseous pressure gradient [3]. Recent study showed that external mechanical loading macroscopically enhanced the intramedulary pressure greatly [4]. The study could strongly support the existence of the micro-anatomical intraosseous fluids in trabeculae. Numerous studies suggest that the stimulus for bone remodeling is mainly caused by the intraosseous fluid flow in trabeculae. Generally, it is called as fluid induced mechanosensation on the connected cellular network. It was reported that bone cells can directly respond to hydraulic pressure in various loading conditions in vitro [5-8]. Also, bone cells exposed to various types of fluid flow in vitro showed to respond with a wide range of biological outcomes [9-25]. Recent ex-in vivo study used the amputated sheep forelimb showed existence of intraosseous fluid flow in compact bone when a 0.2 % of peak strain pulse is applied.[26] This experimental result indirectly showed that there was intraosseous pressure generation to induce the flow. Similarly, Wang et al. [27] found existing interstitial bone fluid flow in bone in vivo. In addition, theoretical models have been suggested and predicted the fluid flow in lacunocanalicular level to support fluid induced mechanosensation on the connected cellular network [28, 29].

As reviewed, the intraosseous pressure generation and fluid flow in lacunocanalicular network of trabeculae could be important to the remodeling process of cancellous bone. However, most of researches related to fluid induced bone remodeling processes are in vitro or theoretical studies. Also, recent in vivo and ex-in vivo studies [26, 27] were qualitative observations. Due to the technical difficulty, experimentally quantifying loading-induced lacunocanalicular fluid flow in vivo has not been feasible. As a result, experimental information about the
amount of intraosseous pressure generation in lacunocanalicular network of trabeculae is never measured. For the purpose, the following question was raised. What could be the maximum intraosseous pressure generation in lacunocanalicular network of trabeculae within the elastic range? To do that, the intraosseous pressure in micro-trabecular specimen was directly measured with a micro-experimental setup representing the most restrictive fluid flow boundaries. Although this experiment performed in vitro condition, the result could let us know the amount of a possible maximum intraosseous pressure generation in lacunocanalicular network of trabeculae within the elastic range.

2. Materials and methods

Figure 1 shows a schematic diagram of the small scale compressive testing machine. The testing machine had a PZT actuator (PI Gmbh, Germany) for the axial loading. The axial loading PZT actuator could load up to 300 N with a full displacement range of 120 μm. When a loading displacement was measured with a 12-bit A/D converter, the resolution of the testing machine was 30 nm. To verify the accuracy of the testing machine, rectangular parallelepiped specimens (500 x 500 x 1000 μm$^3$) using the A6061S-T6 aluminum alloy having the compressive strength of 265 MPa was manufactured and subjected to the compression test. When the aluminum specimens were loaded up to an axial strain of 0.5 %, the measurement error was about 0.2 %. Thus the measurement system was accurate.

Ten microscopic cylindrical trabecular specimens from trabeculae of fresh bovine lumbar vertebrae (Figure 2) were fabricated using the micro-milling machine (EGX-300, Roland, Japan). For the fabrication of the microscopic trabecular specimens, a specially developed micro-router (Figure 3) was used to improve the fineness of surface of specimens. One specimen was obtained from the center of each vertebral body in a cephalad-caudal direction. The specimen size was 500 μm in diameter and 1800 μm in length. To minimize the loss of bone fluid and the damage artifact, these preparation procedures were performed while the specimen was frozen. After fabrication, the specimens were reserved in saline soaked gauze to minimize the loss of bone fluid. Before the experiments, a digital radiograph was taken to exclude specimens with any structural defects.

2.1 Undrained Test

To represent the most restrictive fluid flow boundaries, no flow of bone fluid across the microscopic specimen boundary was permitted. When an external load was applied, deformation in microscopic specimens was allowed in the longitudinal direction (i.e., lateral deformations were totally restricted). For the purpose, a microscopic testing apparatus (Figure 4) was designed specially, and the rigid microscopic stainless steel annulus and solid loading piston provided the prescribed condition by restricting the lateral deformations and the fluid flow. An enamel coating in the groove on the microscopic loading piston prevented leakage of bone fluid during tests. A micro-pressure transducer made by MEMS processing (Figure 5) located at the bottom of the specimen monitored the intraosseous pressure generation. The measurable pressure range was 0 to 100 KPa, and the nonlinearity was 0.5% for the total range of pressure. The micro-pressure transducer had 100 μm x 100 μm of membrane for the measurement. The micro-pressure transducer was directly connected to an amplifier (DA-1602; CAS, Korea). The maximum error of the intraosseous pressure measurement system including the effects of A/D converter resolution (12-bit) was less than 1.0 % full scale.
A microscopic trabecular specimen was positioned in the chamber of the apparatus. In addition, the microscopic piston was inserted into the chamber. Then, the apparatus containing the specimen and piston submerged in a reservoir filled with mineral oil. The reservoir was located in vacuum chamber to remove possible micro-air bubbles in specimens and the chamber of the apparatus. As a result, the specimens and apparatus were saturated by bone fluid and mineral oil. Then the apparatus was mounted on the small scale compressive testing machine. The specimen was subjected to a strain of 0.4% that is reported as less than the elastic range of bone tissues. The loading speed was 9\,\mu\text{m}/\text{min}, which was enough to be considered as a quasi-static condition. The Figure 7 is the completed experimental setup and schematic diagram for the experiment.

### 2.2 Drained Test

Drained tests were performed to compare the results of the previous tests, undrained tests. The same specimens used in the previous tests underwent drained tests in the same experimental setup and using the same testing method except the free flow of fluid in specimens was allowed through the loading piston without coating in the groove.

Figure 8 represents the intraosseous pressure in the lacunocanalicular network in the microscopic trabecular specimens respect to strain. In the undrained condition, the mean compressive stress (±SD) and intraosseous pressure (±SD) at the strain of 0.4% was 28.5 ± 0.82 MPa and 49.2 ± 4.45 KPa, respectively. No intraosseous pressure generation was detected in the drained condition.

**Acknowledgement**

This work was sponsored by the Interdisciplinary Research Grants (R01-2003-000-10798-0) of Korean Science and Engineering Foundation.

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