博 士 学 位 論 文 の 要 約

論 文 題 目： Microstructural analysis of three-dimensional canal network in the rabbit lumbar vertebral endplate using high-resolution micro-computed tomography
高解像度マイクロＣＴを用いた家兎腰椎骨性終板内栄養管の３次元微細構造解析

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Chapter 1: Introduction for this experimental study

Low back pain is one of the most common health problems in the global society. This disease affects 70-85% of the adult population at some time in life, with around 10-20% of people experiencing chronic low back pain. The total annual medical costs for work-related low back-pain were 82.14 billion yen that accounted for 9.8% of the entire medical costs in Japan. Intervertebral disc (IVD) degeneration has been considered as a major cause of back pain. Although the multiple components link with disc degeneration, including age, genetic inheritance, heavy lifting and smoking, its etiology and pathogenesis remain unclear. Because the IVD is the largest avascular tissue in the body, decreased or impaired nutrient supply into the intervertebral disc caused by cartilage endplate calcification and vertebral endplate sclerosis, has been considered as one of the important contributors for disc degeneration. The previous studies have confirmed the presence of the canal structure in the vertebral endplate by histology, angiography and electron-microscopy. However, microstructure of three-dimensional (3D) canal network in the vertebral endplate remains poorly understood.

Chapter 2 and 3: Hypothesis and purpose of this thesis

Based on previous observations on the vertebral endplate canal structure, we hypothesized that microstructure of 3D canal network in the vertebral endplate can be characterized through the canal size, orientation and depth. The purpose of this study is to analyze length, diameter, orientation angle and depth of the individual canals in the normal rabbit lumbar vertebral endplate using a high-resolution micro-computed tomography (μCT).

Chapter 4: Description of the methods used in this thesis

The lumbar spines (L1-L6) from five adult Japanese white rabbits (Shimizu Laboratory Supplies Co, Ltd, Kyoto, Japan) were isolated. A total of 25 bone-disc-bone (BDB) units were prepared by cutting parallel to the vertebral bodies, including an IVD with adjacent vertebral endplates, and the 6 mm diameter samples from the central region of the BDB units were obtained. One cranial endplate surface to IVD (caudal endplate of cranial vertebra) and one caudal endplate surface to IVD (cranial endplate of caudal vertebra) were simultaneously imaged using a high-resolution μCT scanner (SMX-160CTS, Shimadzu Co., Kyoto, Japan; maximum resolution: 0.8 μm) with 1.4×1.4×1.8 μm voxel size. 3D canal network models in the vertebral endplate and 3D surface models of vertebral endplates were created, and the individual canals were segmented for microstructural analysis. Both the 3D endplate surface and the individual canal models were further converted to the point-cloud data sets, respectively.

The individual canal angles between the normal vector to the vertebral endplate towards the IVD and the eigenvector of each canal directed longitudinal direction of the canal were calculated by dot product, and each canal was separated into the longitudinal and the transverse canals by orientation angle bimodal distributions. The mean canal diameter was determined by volume/length. Depth of the individual canal was determined by analyzing the least distance between the centroid of each canal and surface of the vertebral endplate in the normal direction for the vertebral endplate.

The canal length and diameter were compared between the longitudinal canal and the transverse canal using unpaired t-tests. Ward's clustering analysis was used to categorize the individual canals based on distribution of canal diameter and angle or combinations of length and depth. Chi-square test was used to assess distribution of angles among the clusters. Data are presented as mean ± standard deviation (SD).

Chapter 5: Results of this thesis

Canal angles followed a bimodal distribution and could be separated into two distinct canal orientations; ***the longitudinal canal*** ($0^\circ \leq \theta < 70^\circ$ and $110^\circ < \theta \leq 180^\circ$) and ***the transverse canal*** ($70^\circ \leq \theta \leq 110^\circ$). The longitudinal and the transverse canals accounted for 58.3% and 41.7% of all canals, respectively, and the mean inclinations of them were 47.7±26.4° and 87.3±10.0°, respectively. The mean length and diameter of the longitudinal canals were 87.3±57.7 μm and 47.4±17.0 μm, respectively, and those parameters of the transverse canals were 117.2±88.4 μm and 54.9±27.2 μm, respectively. The transverse canals were significantly longer and wider than the longitudinal canals ($p<0.0001$).

Based on Ward's clustering results, ***the large-scale transverse canals*** running parallel to the vertebral endplate were detected, which were oriented at 92.5±8.8° from the vertebral endplate normal direction, and the mean length and diameter were 245.3±129.9 μm and 152.1±24.3 μm, respectively. The canals connected to the bone marrow space were termed as ***the marrow-contact canals***, and the mean length, diameter and angle of which were 125.5±85.5 μm, 58.0±22.4 μm, and 59.5±30.6°, respectively. Additionally, the canals, existing in the surface region between the large-scale transverse canals and the vertebral endplate surface, were categorized into three groups using Ward's clustering; ***the surface-surface canal***, ***the surface-middle canal*** and ***the surface-deep canal***, respectively. The surface-surface, the surface-middle, the surface-deep, the large-scale transverse and the marrow-contact canals were located at 76.2±33.7 μm, 115.6±43.5 μm, 189.9±43.6 μm, 224.1±62.7 μm, and 263.1±90.9 μm from the endplate surface, respectively. A histogram of the surface-middle canal angles showed the large peak at 80°-90°, while the surface-surface and the surface-deep canal inclinations followed multimodal distributions. Chi-square tests showed significant differences in distribution of canal inclination among these canal sub-categories ($p<0.0001$).

Chapter 6: A series of discussions

A high resolution μCT technique used in this study was able to evaluate 3D canal network in the normal rabbit lumbar vertebral endplate. In addition, the individual canals present inside the vertebral endplate were isolated and quantitatively characterized by measuring their length, diameter, orientation angle and depth from the endplate surface. The results revealed differences in the canal structure above and below the large-scale canals running parallel to the endplate surface that connected to not only the vertebral endplate surface but also the vertebral endplate surface through the vertically oriented canals. Such structure may play an important role for collecting and redistributing the fluid within the endplate, the exchange of nutrients and metabolites.

Furthermore, the quantitative assessment of the canals present between the vertebral endplate surface and the large-scale transverse canals indicates the possibility of which the Haversian system exists in the vertebral endplate, and information of such canals will provide us the insight on insufficient nutrient supply into the IVD associated with disc degeneration.

Chapter 7: Conclusion of this thesis

Using a high-resolution μCT technique, 3D canal network in the rabbit lumbar vertebral endplate revealed the distinct depth-dependent structure that characterized through length, diameter and orientation of the individual canals in the quantitative manner. Although the μCT technique only allows us to analyze the bony structure, the interconnected canals serve as the conduit for vessels in the vertebral endplate. Our findings in the present study could provide essential information to understand nutrient pathways through the vertebral endplate.