博士学位論文審査要旨

２０１４年　２月　１４日

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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要　　旨：

椎間板は生体内で最も大きい無血管組織であり、椎間板内の代謝を担う椎間板細胞への主たる栄養供給は、椎体から骨性終板を貫通する管（栄養管）内に存在する毛細血管や洞様血管を通して行われている。骨性終板内の栄養管狭小化および石灰化は、椎間板への栄養供給を低下させて細胞外マトリックスの代謝破綻を促し、椎間板変性を招いていると推察されている。しかし、骨性終板内栄養管の3次元微細構造は未だ明確にされていない。そこで本論文では、高解像度マイクロCT技術を用いて家兎腰椎骨性終板内栄養管の3次元微細構造解析を行い、その多層構造について詳細に議論した。

第1章、第2章、第3章では、骨性終板内栄養管の研究についての背景、本研究の仮説および目的について述べられている。

第4章では、骨性終板内の3次元栄養管モデルおよび3次元終板表面モデルを用い、栄養管の配向、長さ、直径、椎体終板表面からの深さをパラメターとして3次元微細構造解析を行った。

第5章では、第4章で述べた3次元微細構造解析の結果を示した。骨性終板内栄養管の配向分布は、垂直方向と水平方向にピークを持つ二峰性を示し、水平方向栄養管の長さ・平均直径が垂直方向栄養管よりも大きいことを明らかにした。また、各栄養管の3次元微細構造結果をもとにクラスター分析を行うことで、その多層構造を定量的に分類することができた。

第6章では、第5章で定量的に分類された栄養管群がそれぞれどのような役割を担っているかを議論し、その多層構造が骨性終板内の血管構造に起因していることが示唆された。

第7章においては、本研究で用いた3次元微細構造解析に対する結論を述べた。

以上より、本論文は高解像度マイクロCTを用いて家兎腰椎骨性終板内栄養管の3次元微細構造を明らかにし、その多層構造を定量的に評価した。本論文で得られた結果は、栄養管狭窄と椎間板変性の関係を明らかにする上で重要な基礎データになる。よって、本論文は、博士（工学）（同志社大学）の学位論文として十分な価値を有するものと認める。

総合試験結果の要旨

２０１４年　２月　１４日

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副査：同志社大学大学院生命医科学研究科　教授　小泉　　範子

要　　旨：

論文提出者は、2011年4月に同志社大学大学院生命医科学研究科生命医科学専攻博士課程（後期過程）学生として入学し、脊椎を中心とした運動器疾患の病因解明および再生医学的手法を用いた治療法の開発に関する研究を行った。各年度において優れた成果を挙げ、その内容は国際学術誌に6編すでに掲載済みである。本論文の主たる内容は、Journal of Orthopaedic Researchに投稿され、現在審査中である。

2014年1月11日（土）午後3時より約1時間15分にわたって提出論文に対する学術講演会（博士論文公聴会）が開催され、活発な質疑応答がなされたが、いずれも提出者の説明により十分な理解が得られた。さらに、公聴会終了後、審査員により論文内容ならびにこれらに関連する諸問題について口頭試問を実施した結果、いずれも十分な学力を有することを確認した。

論文提出者は、本研究科修了に必要な所定の単位を修得している。また、論文提出者は、英語の語学試験に合格し、2011年8月～2012年7月には日本学術振興会若手研究者戦略的海外派遣事業（脳循環を活性化する若手研究者海外派遣プログラム）の派遣研究員としてカリフォルニア大学サンディエゴ校（UCSD）医学部整形外科で研究を行ってきたことから、英語力についても十分であると認められる。

よって、総合試験の結果は合格であると認める。

博 士 学 位 論 文 要 旨

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

氏　　名： 山口　知紀

要　　旨：

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.