

## Study of the Peripheral Vasculature of the Intervertebral Disc in Rats

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### ■ Introduction

Researchers hypothesized that smoking is a risk factor of back pain. Many patients who had back pain were smokers. In addition, statistics showed that former and current smokers often experienced more pain and experienced pain more frequently than non-smokers. Therefore, smoking has an effect on back pain in general. However, how smoking precedes back pain was not well understood.

On the other hand, nicotine induces blood flow reduction, hypoxia, chemical and morphological changes in the intervertebral disc (IVD)<sup>1, 8</sup>. Little information was on mechanism how nicotine induces disc degeneration. One possibility was that nicotine causes changes to vessels of peripheral vasculature of the IVD. The nutrients are transported to the IVD by two routes. One is from the capillary beds above the subchondral bone to the nucleus and inner annulus of the disc by diffusion<sup>2</sup>. Another is direct supply from the peripheral vasculature to the outer annulus. Both routes are supported by peripheral vasculature, although route one is not considered a direct route<sup>2</sup>. Therefore, impairment of the peripheral vasculature could limit the supply of nutrients, in which poor supply of nutrients will lead to disc degeneration.

To understand relationship between changes in vessels and impairment of peripheral vasculature of IVD, some studies focused on studying the inner layer that is formed by endothelial cells (EC) of vessels. Inwahashi's histology data illustrated the enlargement of vascular EC and narrowing of the vascular lumen in nicotine models. Thus, potential change of vessel diameter exists. On the other hand, vessel diameter is a quantitative parameter that may provide evidence of changes induced by nicotine to vessels.

In this study, we approached the relationship between back pain and smoking from examining how nicotine impairs the peripheral vasculature of the intervertebral disc so to induce back pain. We specifically aimed to develop and refine methods to visualize three-dimensional structure of the vasculature in the periphery of the intervertebral disc in rats, due to not many studies did had done so. We were also interested in comparing diameter of vessel from a smoking and non-smoking model to obtain quantitative results.

### ■ Materials and Methods

Fourteen Sprague Dawley rats (9 month-old) were assigned to two groups of (n=7), control and treatment. Animals received subcutaneous injection of saline and nicotine injection. Treatment group received 1.2mg nicotine by mini-osmotic pump (alzet® model 2004, 25µL/hr) per day for 8 weeks. Control group received saline under the same conditions. Eight weeks after experiment, microfil was injected in all rats by perfusion and then spines were harvested.

Using microCT scanner Skyscan 1076, samples were then scanned at 9 microns in UCSD. 70kV was used for scanning of all samples. After scanning, all raw data was reconstructed with matrix size approximately 2500x2500, depending on the selected region of interest and size of samples. Before importing data to create three-dimensional model in Mimics, reconstructed data was segmented to L4 level using CTAnalyzer (1.11.42+ ©2003-11 Skyscan) and rotated using DataViewer (1.3.3 ©Skysan).

Images were then imported to Mimics 14.11 ®Materialise to create three-dimensional models of the peripheral vasculature of the intervertebral disc. Two methods of creating three-dimensional mask of peripheral vasculature of the IVD were tested in this experiment, due to the

fact that few three-dimensional models had been constructed in previous studies. The first method was to over-threshold data, so that all vessels were selected and being included in two-dimensional mask along with bone. The second method was to manually create mask of vessel slide-by-slide.

Two methods to calculate vessel diameter of both control and treatment group were also tested. The first method was using the volume of the mask of vessel and length of vessel that was measured by measurement tool, distance over surface, in Mimics. Assuming vessel was cylindrical in shape, we calculated vessel diameter using relationship between diameter, length, and volume.

$$\text{Volume} = \pi \times \left( \frac{\text{diameter}}{2} \right)^2 \times \text{length}$$

The second method was using a customized program written by Dr. Nozomu Inoue. This program utilized point cloud data of three-dimensional mask and centerline to calculate vessel diameter, including minimum, maximum, and average.

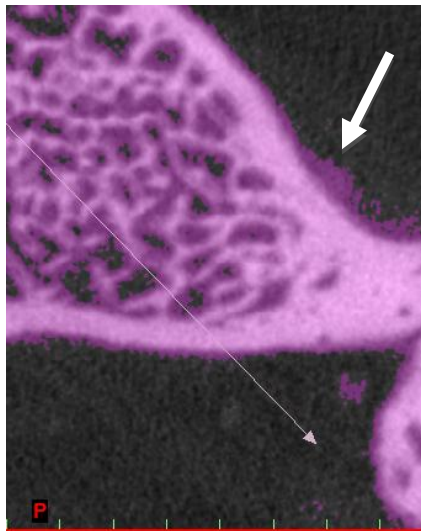


Figure 1. Over-threshold data so to include vessels along with bone in two-dimensional mask in Mimics.

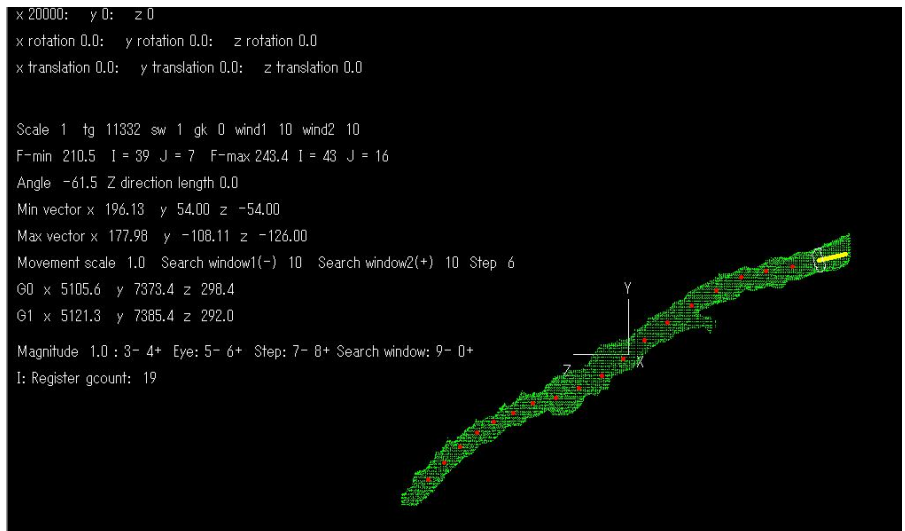


Figure 2. Program written by Dr. Nozomu Inoue utilizing centerline to calculate minimum, maximum, and average diameter of vessel of peripheral vasculature of the intervertebral disc.

## ■ Results

Peripheral vasculature of the intervertebral disc of both control and treatment appeared in shape of an up-side-down “V.” Intersection point of the “V” appeared connected in all analyzed control groups. However, intersection points were shown disconnected in analyzed treatment groups with one exception (nicotine#07). However, whether over-thresholding or manual drawing is a better method to create two-dimensional mask of vessels remained unclear. We also observed vessel entering the intervertebral body, but visibility of entrances varied depending on quality of microfil injection. On the other hand, we observed the indirect route that transports nutrients to the nucleus and inner annulus in addition to the direct route that transports nutrients to outer annulus.

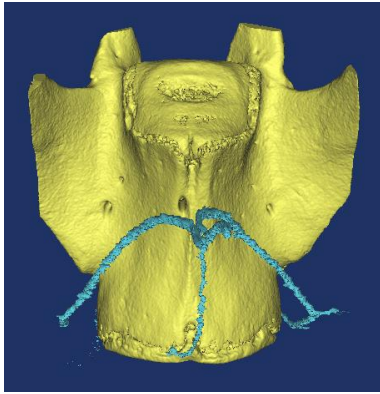


Figure 3. “V” shape vessel of sample control#04.

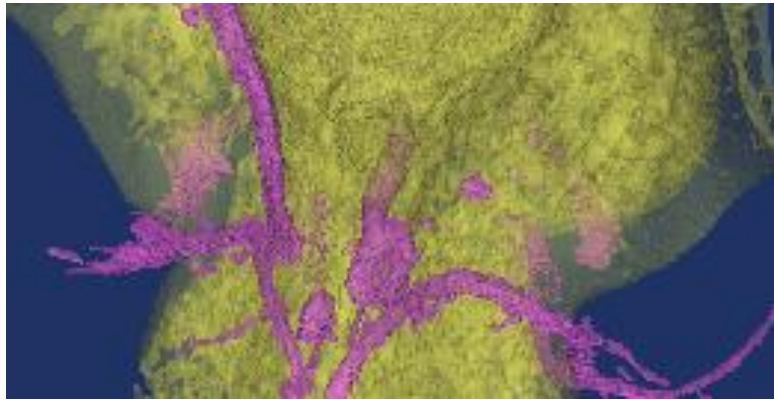


Figure 4. Vessel entering the intervertebral body of sample nicotine#07.

Calculation of vessel diameter was performed on a selected section of “V” shape vessel from one control and one treatment sample. The dimension of threshold region of vessel represented dimension of vessel lumen, in which diameter of threshold was the inner diameter of vessel. Calculated diameters of control and treatment group using two methods were similar. However, significance of two methods was not clear.

#### ■ Discussion

To understand the relationship between smoking and back pain, we established study to observe changes induced by nicotine to the peripheral vasculature of the intervertebral disc in rats. We constructed three-dimensional models of the peripheral vasculature and define landmarks of the peripheral vasculature. Morphological difference, whether intersection of “V” shape vessel is connected or disconnected, hinted effect of nicotine to the nutrients transportation routes. Similar diameters of control and treatment group did not provide representative evidence that nicotine either expands or narrows the vascular lumen.

Two methods, over-thresholding and manual creation of vessel mask, were tested to construct three-dimensional models. Method of over-thresholding lowered the possibilities missing small vessels in the peripheral vasculature. However, much noise was included in mask. Manual creation of mask was time-consuming, but provided a considerable accurate three-dimensional model without creating noise. Quantitative analysis is needed to compare the significance of two methods. For example, using customized volume merge program to compare the volumes of three-dimensional masks.

Two methods were also used to calculate diameter of vessels in the periphery of the intervertebral disc. Considering quality of microfil injection affects visibility of vessel in microCT scanning data, we performed calculation on selected section of vessel. More data needs to be analyzed before establishing a conclusion whether one method provides more significant results than the other.

In this study, we gained an overview of the structure of the peripheral vasculature of the intervertebral disc in rats, defined landmarks on these created structures of both control and treatment groups, and established methods to calculate vessel diameters. Although no significant data showed one method is better than the other in creating three-dimensional model of

peripheral vasculature of the intervertebral disc and calculation of vessel diameter, analysis in the following step is clear. We look forward to quantify and define significant method on creating three-dimensional model of peripheral vasculature of IVD, calculation of vessel diameter, and gain understanding of morphological difference in peripheral vasculature of the IVD between control and treatment group.

#### ■ References

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