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TITLE: A Novel Method to Visualize the Three-dimensional Alteration of Microvasculature in a Cord Injury Model: A Study with Synchrotron Radiation Micro-angiography

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ABSTRACT BODY:

Introduction: Acute spinal cord injury (SCI) is a devastating traumatic event involving the central system. The trauma which causes an initial damage to the vasculature will aggravate a secondary cord injury. Investigation of the three-dimensional (3D) pathologic changes of microvasculature conformation after SCI is important for better understanding of the subsequent pathological processes. The aim of our study was to develop a novel methodology and assess the feasibility of the synchrotron micro-computed tomography (SRμCT) combined with micro-angiography to visualize the 3D alteration microvasculature and obtain the morphometry data in a rat SCI model.

Methods: All experiments were conducted in compliance with the guidelines established by the and Use Committee of the Central South University. (1) Sample Preparation: A total of 10 Sprague-Dawley rats were utilized. Five SD rats underwent laminotomy at 10th thoracic cord level and subjected modified Allen’s weight impacting to induce an incomplete SCI model. The other five rats only underwent laminotomy and served as the control group. 24 hours post-injury, all rats were subjected to angiography left ventricle with X-ray contrast agent (Microfil MV-122, Flow Tech, CA, USA). The samples were dehydrated with graded ethanol and immersed in methyl salicylate for 12-24h. (2) Image Acquisition: Microangiography was performed at the BL13W1, the x-ray imaging and biomedical application the Shanghai Synchrotron Radiation Facility (SSRF) in China. The sample was longitudinally placed centre of rotary stage allowing a range of 0°-180° rotation. The scanning energy was set at 15.0KeV. The sample-to-detector distance was 3 cm. The time of exposure was established to 2 sec. The resolution was 3.7μm. A total of 720 initial projecting photos were captured. All these projecting transformed into digital slices using the filtered back-projection algorithm reconstruction software by BL13W experimental station). The fine 3D blood vessels on full scale of the spinal cord were
VG Studio Max 3D reconstruction software (version 2.1, Volume Graphics GmbH, Germany). After by the SRµCT, all the samples were selected for histo-morphological thick section examination stereomicroscope. And the comparative analysis of neurovascular morphology was performed histological sections and 3D X-ray absorbed images. The vascular morphology was calculated vascular volume analysis model of VG Studio Max software.

**Results:** (1) The 3D morphology of the normal spinal cord vasculature could be vividly visualized micrometer scale by microangiography with SRµCT (Fig. 1a). The reconstructed images delineated shape, branches and spatial distribution of the intrinsic arteries of the spinal cord, which included intramedullary and extramedullary microvessels. The vasculature of the spinal cord in the white not served by the capillary beds when compared to the abundant capillary in the gray matter. Where gray and white matter met, the capillary beds were denser than those in the white matter (Fig. characteristics of vascular arrangements in 3D X-ray absorbed images were well matched with histological sections (Fig. 1d & e). (2) The 3D morphology of microvasculature in spinal cord at post-injury: Although the large vessels on the surface of the spinal cord, such as the pial arterial the anterior spinal artery(ASA) could still be visualized, most of the intramedullary micro-vasculature injured cord decreased dramatically and formed an avascular area. Additionally, the damage area only concentrated in the central grey matter, it but also extended rostrally and caudally from the epicentral zone (Fig. 2a,b & c). These pathological features could also be visualized in the specimen scanned by stereomicroscope (Fig. 2d & e). (3) Illustrate the change in the micromorphology data The volume of vasculature (Vv) in the injury epicentral zone was dramatically decreased when the normal blood supply of the spinal cord (Fig. 3b).

**Discussion:** In this study, we successfully delineated the 3D morphological features of microvasculature normal and injured spinal cord segment using SRµCT based micoangiography for the first time. of this investigation demonstrated that trauma is the direct factor, which causes the severe destruction microvascular architecture. The region of the spinal cord injury was not confined to epicentral zone would extend rostrally and caudally along the central tube with a spindle shape.

**Significance:** These findings indicate that the combination of synchrotron radiation with microangiography as a novel imaging technique can be used to explore the regeneration process of the microvascular structure after acute SCI in a rat model. Compared to the conventional method, the 3rd generation synchrotron radiation, which is characterized by high brilliance allowing a 3D visualization of the micro-vessels, has the potential to visualize the pathologic changes of microvasculature in neurovascular disorders, and evaluate the efficacy of drug therapy in the experimental study.

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Fig. 1. Microangiography image of rat normal spinal cord vasculature. The high-resolution 3D images normal spinal vascular structure by SRμCT (a, b & c). The histological section stereograms by stereomicroscope (d & e). The entire 3D vascular framework (a). The transverse section view of subregion(b & d) outlined in white circle (a). The details of microvasculature in gray matter are observed by the enlarged imaging (c & e). At this view, the 3D X-ray absorbed images well matched histological sections date. Bar=200μm (a, b & d). Bar=100μm (c & e).

Fig. 2. Hierarchical investigation of the alteration of microvasculature network in SCI 24 hours post-injury. The entire 3D vascular framework after injury (a). Images detect by SRμCT (b & c). The histological images (d & e). Z-buffer slice of the section (b) marked in white circle (a). Surface rendered histological section image of the same subregion (d). The enlarged images of the avascular region (c & e) rectangle (b & d) suitable for visualization of the details in spinal cord injury segment. Bar=200μm Bar=100μm (c & e).

Fig. 3. The quantitative parameters (volume of vasculature, Vv) of vascular micro-morphology The dashed ellipses highlight the injury part; b: The Vv in the injury epicentral zone was decreased dramatically.