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A perfusion procedure for imaging of the mouse cerebral vasculature by X-ray micro-CT☆

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HIGHLIGHTS

- Brain samples perfused with contrast agent have high inconsistency in the filling of the posterior cerebral circulation.
- We revised the Microfil perfusion protocol, in order to reduce the variability of the outcome in samples.
- The Microfil is first perfused through the posterior circulation by blocking the flow to the anterior circulation.
- A workflow is provided to verify the successful completion of each surgical step.
- The cerebellum shows 6.9%, and the midbrain about 8.7% increase in the percentage of vessel segments.

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ABSTRACT

Background: Micro-CT is a novel X-ray imaging modality which can provide 3D high resolution images of the vascular network filled with contrast agent. The cerebrovascular system is a complex anatomical structure that can be imaged with contrast enhanced micro-CT. However, the morphology of the cerebrovasculature and many circulatory anastomosis in the brain result in high variations in the extent of contrast agent filling in the blood vessels and as a result, the vasculature of different subjects appear differently in the acquired images. Specifically, the posterior circulation is not consistently perfused with the contrast agent in many brain specimens and thus, many major vessels that perfuse blood to the midbrain and hindbrain are not visible in the micro-CT images acquired from these samples.
New method: In this paper, we present a modified surgical procedure of cerebral vasculature perfusion through the left ventricle with Microfil contrast agent, in order to achieve a more uniform perfusion of blood vessels throughout the brain and as a result, more consistent images of the cerebrovasculature. Our method consists of filling the posterior cerebral circulation with contrast agent, followed by the perfusion of the whole cerebrovasculature.
Results: Our histological results show that over 90% of the vessels in the entire brain, including the cerebellum, were filled with contrast agent.
Comparison with existing method: Our results show that the new technique of sample perfusion decreases the variability of the posterior circulation in the cerebellum in micro-CT images by 6.9%.
Conclusions: This new technique of sample preparation improves the quality of cerebrovascular images.

1. Introduction

The imaging of blood vessels is a common method to acquire information about the development of vascular systems and their connectivity, the blood perfusion into different organs, and the pathology of vessels (van den Wijngaard et al., 2013; Young et al., 1979; Pathak et al., 2008). Ultrasound, magnetic resonance angiography (MRA) and X-ray angiography are common methods to image vessels and are useful for calculating the blood perfusion and finding pathologies in the vessel walls. In recent years, micro-CT imaging has been utilized to image small animals such as mice for vascular biology research (Jorgensen et al., 1998; Holdsworth and

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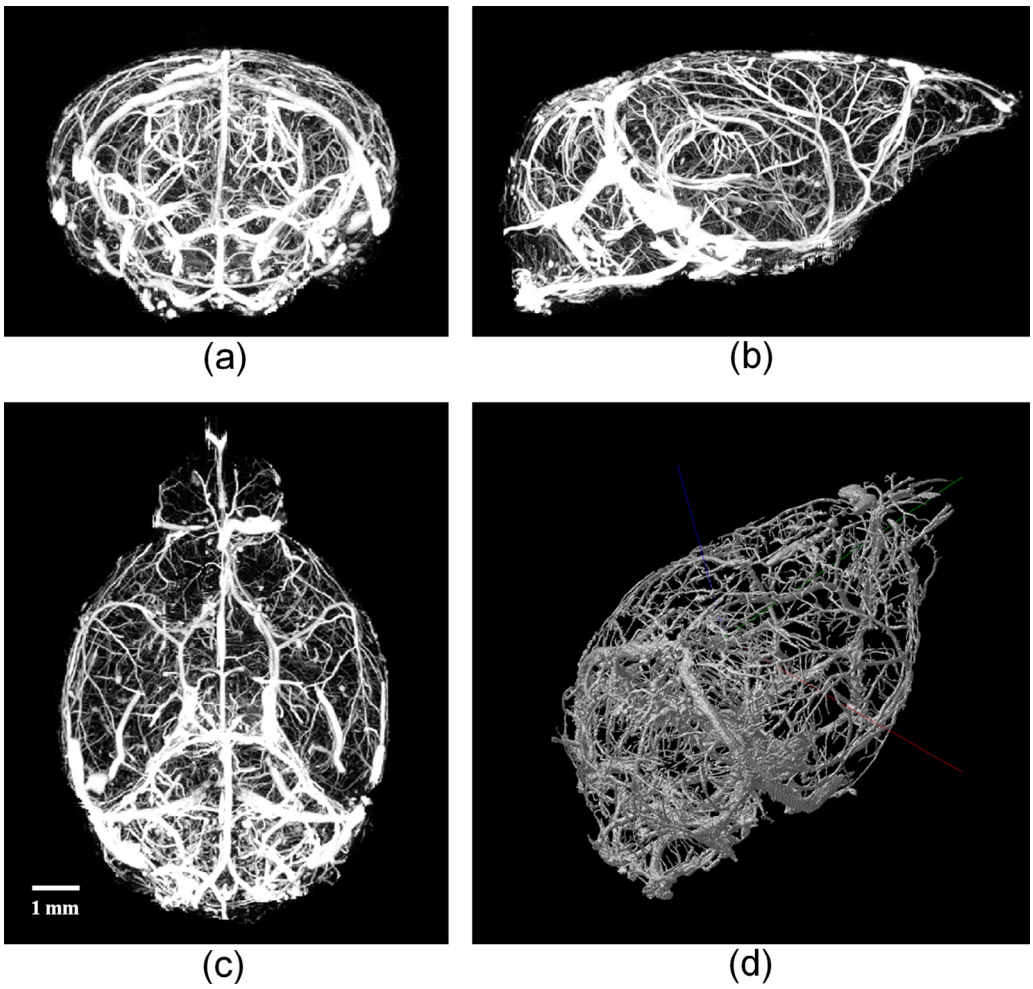

Fig. 1. Maximum intensity projections (MIP) and 3D rendering of the micro-CT image of a wild-type C57BL/6 mouse brain which was perfused with Microfil using our new method of contrast agent perfusion and was imaged with 20 μm isotropic resolution. (a) Coronal, (b) sagittal, and (c) axial MIP representation, and (d) the 3D rendering.

Thornton, 2002). Due to its high resolution and short scan time, micro-CT is a suitable imaging method for imaging microvasculature of intact rodent organs. The use of low viscous CT contrast agents that fill the lumen of vessels has made it possible to acquire high-resolution 3D images of vascular systems such as kidney and placenta (Bentley et al., 2002; Nordsletten et al., 2006; Marxen et al., 2006; Rennie et al., 2007; Yang et al., 2010). The cerebrovascular casting in mouse using casting agents can be used to image the brain sample with bright field microscopy, stereomicroscopy, scanning electron microscopy, as well as micro-CT (Walker et al., 2011; Cruise et al., 2009; Krucker et al., 2006; Heinzer et al., 2006). Specifically, Microfil contrast agent, a low viscous silicone rubber injection compound containing lead pigments, has been used to perfuse the vascular system to be imaged *ex vivo* with micro-CT (van den Wijngaard et al., 2013; Chugh et al., 2009; Rennie et al., 2010; Chutkow et al., 2002; Daneyemez, 1999; Vasquez et al., 2011; Marxen et al., 2004). In this perfusion method the lumen of vessels are filled with contrast agent. The lead pigments in the Microfil provide high contrast compared to the background tissue needed to acquire a complete high resolution 3D image of the vascular structure. The perfusion technique presented here is relatively easy to perform and can be used to visualize the whole vascular system. In addition, the perfused brain samples can be embedded in paraffin and be used for histological sectioning in order to examine the cells and tissues, for example to stain and visualize the endothelial cells in the vessel walls. As an example of value of this technique, Fig. 1 shows the sagittal, coronal, and axial maximum intensity projections (MIPs) representation of a wild-type C57BL/6 mouse brain perfused with contrast agent and imaged with 20 μm isotropic resolution. The arteries, veins and sinuses in mouse brain can be identified on the 3D high resolution micro-CT image.

A retrospective analysis has been performed on previous studies from our group. This analysis showed that despite the high resolution 3D images that can be acquired from contrast agent perfused cerebral arteries and veins and the high density of the visible cerebral vessels in the micro-CT images, the connectivity and branches of cerebrovascular structure showed high variations, especially in the cerebellum area. We hypothesize that the variations and artifacts introduced by the perfusion procedure can be the cause for differences among images of different subjects. Fig. 2 shows the sagittal view MIP representation of micro-CT images taken from two wild-type C57BL/6 mice. A MIP representation of a 2 mm slab centred at the mid-sagittal plane for the samples are also shown in the right column of Fig. 2. As it can be seen from this figure, some major vessels, such as arteries perfusing the cerebellum, are completely or partially missing from the micro-CT image shown in the second row. This difference in the images of the two samples is not likely to be caused by underlying physiological differences. The two samples share similar genetic background and environment. Also, missing major arteries in the brain, as can be seen in the second row of Fig. 2, would likely have led to premature death or a severe behavioural deficit. Since the imaging protocol is also consistent among all the samples, the likelihood is low of the variations originating from the instrumental sources.