Journal of
Synchrotron Radiation

ISSN 0909-0495

Received 29 November 2009
Accepted 20 May 2010

Microvascular imaging using synchrotron radiation

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In vascular diseases, the involvement of small vessels can be very crucial physiologically. Morphological changes of vasculature and alterations may be promising characteristic criteria for investigating disease progression and for evaluating therapeutic effects. Visualization of microvasculatures is an important step in understanding the mechanism of early vessel disorders and developing effective therapeutic strategies. However, the microvessels involved are beyond the detection limit of conventional angiography, *i.e.* 200 μm. Thus, faster and higher-resolution imaging technologies are desired to capture the early anatomical structure changes of vasculatures in study of the disease. A new angiography system, synchrotron radiation microangiography, has been developed in this study. It allows for enhanced sensitivity to contrast agents and superior image quality in spatial resolution. Iodine and barium sulfate were used as blood vessel contrast agents. Physiological features of whole-body mouse microvasculature were investigated using synchrotron radiation for the first time. The intracranial vascular network and other blood vessels were observed clearly, and the related anatomy and vessel diameters were studied. Dynamic angiography in mouse brain was performed with a high spatial image resolution of around 20–30 μm. Future research will focus on the development of novel specific targeting contrast agents for blood vessel imaging *in vivo* with a long half-life and fewer side effects.

Keywords: microvascular; imaging.

1. Introduction

In some circulatory disorders of the brain, heart and other organs, the involvement of small vessels is very crucial physiologically. *In vivo* animal models of tumor, stroke, subarachnoid haemorrhage and other diseases are commonly used to study how morphological changes of vasculature and alterations involved in the disease progress can be used in evaluating therapeutic effects (Schambach *et al.*, 2010). Visualization of microvasculatures is an important step in understanding the mechanism of early vessel disorders and developing effective therapeutic strategies. Faster and higher-resolution imaging technology is highly desired in order to capture the anatomical structure information of vasculatures in the development of these diseases, and to guide therapeutic strategies. However, small vessels, including microvasculature involved in early brain diseases and tumor angiogenesis, are beyond the detection limit of conventional angiography.

Conventional angiography is commonly used to evaluate *in vivo* vasculatures of diameter >200 μm (McDonald & Choyke, 2003; Fink *et al.*, 2003; Myojin *et al.*, 2007; Cai *et al.*, 2008). Mammography, which has the highest spatial resolution in clinical practice, does not have sufficient resolution to visualize small vessels of less than 50 μm in diameter (Kuzmiak *et al.*, 2005). New imaging modalities such as magnetic resonance angiography and computerized tomographic angiography have been developed. They provide excellent tools for vascular visualization, but the spatial resolution is still a remaining issue (Kelly *et al.*, 2007). In addition, information regarding the flow dynamics of the circulation can be obtained when contrast agents are injected during angiography. This is particularly important to the study of therapeutic strategies because a treatment plan is highly dependent on the information obtained through those dynamic images. Therefore, development of intravenous angiography would significantly benefit patients with vascular pathology using a safe, dynamic and high-resolution system.

Recently, *ex vivo* and *in vivo* microangiography using synchrotron radiation combined with a high-resolution and high-speed imaging system has provided a powerful tool to study small vessels of various animal organs (Mori *et al.*, 1996). Detection of microcirculation in the dermis and subcuta-

neously implanted tumors is becoming possible. Tanaka *et al.* (1999) were able to visualize coronary vessels (50 μm) in exposed dog heart. Moreover, Yamashita *et al.* (2002) used synchrotron radiation to study mouse coronary arteries in beating hearts *in vivo*, and Umetani *et al.* (2007, 2009) and Tokiya *et al.* (2004) were able to observe the microvasculature of tumors in rabbit auricle (*in vivo*), *i.e.* vessel diameters as small as 20–30 μm. Recently, some reports showed that intracranial arteries of diameter 10 μm could be observed by synchrotron radiation microangiography (Myojin *et al.*, 2007; Umetani *et al.*, 2007; Kidoguchi *et al.*, 2006; Morishita *et al.*, 2006). Based on these observations, we developed a micro-angiographic system using synchrotron radiation and have investigated physiological features of whole-body mouse microvasculature for the first time at the newly built Shanghai Synchrotron Radiation Facility (SSRF), a third-generation synchrotron radiation facility. Our goal is to demonstrate the possibility of studying the early vascular diseases and tumor angiogenesis using dynamic synchrotron radiation micro-angiography in the near future.

2. Materials and methods

2.1. Preparation of contrast agents

For high-contrast images of microcirculation, the contrast agents used were iodine (Omnipaque 350) and barium sulfate. Barium sulfate was obtained through the reaction of barium chloride and sulfuric acid. Because barium sulfate particles were usually 1–100 μm in diameter and most of them could hardly pass through vessels of a few micrometres, the precipitation was filtered using a vacuum pump and a filter membrane with holes of diameter 5 μm. The filtered barium sulfate was then centrifuged at 3000 rev h^{-1} rotation speed for half an hour and particles were separated from supernatant. After washing, barium sulfate was suspended in glycerol (50% water solution) to form a solution with a concentration of 1 g ml^{-1}.

2.2. Injection of contrast agents

Female BALB/c nude mice (20 ± 2 g, 5 weeks) were bought from the Animal Center, CAS, Shanghai, China. They were housed in isolated cages with a 12 h light/dark cycle, and fed with sterile food. They were anesthetized using sodium pentobarbital intraperitoneally. All experimental animals were handled following the guidelines provided by the Animal Welfare Committee at the Shanghai Sixth People's Hospital, affiliated to Shanghai Jiaotong University.

For brain imaging, contrast agents were injected through the jugular vein. The mouse was held stable on a frame with a specially shaped head holder. Angiography was performed with an automated injector that was programmed to deliver 0.2 ml s^{-1} of non-ionic contrast media containing 40% iodine (Omnipaque 350) for 2 s. Serial images of the mouse vessels were then recorded. The frame was then fixed vertically to the X-ray beam to obtain axial views of the cerebral arteries. For imaging microvasculature in the whole body, the vena cava superior was cannulated with the tip of the injector (0.5 mm outer diameter) *via* which barium sulfate was injected and the mouse was sacrificed before imaging.

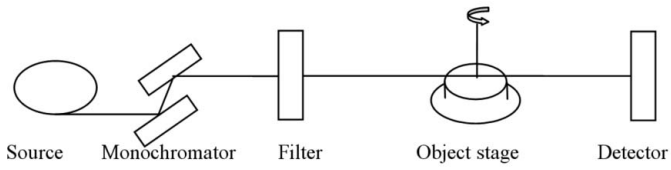

Figure 1
Schematic of the experimental set-up for imaging using monochromatic synchrotron radiation X-rays. The photon energy of the X-rays is selected by tuning the double-crystal monochromator.

2.3. Full-field imaging with synchrotron radiation X-rays

All samples were imaged at beamline BL13W of the SSRF in China. The SSRF is a third-generation synchrotron source with a 200–300 mA beam current and 3.5 GeV storage energy, now the fourth highest in the world. Beamline BL13W of the SSRF is designed to provide a photon energy ranging from 10 keV to 60 keV and a beam size of 45 mm (horizontal) × 5 mm (vertical) at the object position at 20 keV. Samples are placed approximately 30 m downstream of the source, while the detector distance ranges from 0 to 8 m. An experimental set-up for imaging using monochromatic synchrotron radiation X-rays is shown schematically in Fig. 1.

The samples are mounted on the translation/rotation stage controlled by a precise step motor which provided a maximum translation distance of 5 cm along each axis. The detection system consists of a thin (100 μm) $CdWO_4$ cleaved single-crystal scintillator and a CCD camera. The equivalent detector resolution is ∼13 μm. The distance from the sample to the detector could be precisely controlled using a motor. For brain imaging, the imaging parameters used were: energy, 34 keV; sample–detector distance, 1 m; exposure time, 50 ms $frame^{-1}$. For whole-body mouse microvasculature imaging, the parameters were: energy, 37.6 keV; sample–detector distance, 1 m; exposure time, 10 ms $frame^{-1}$.

3. Results

Figs. 2(*a*)–2(*h*) show the synchrotron radiation X-ray dynamic angiography process in the mouse brain when iodine was injected as contrast agent. Because of the limited beam size, the whole brain blood vessels could not be observed at the same time. In Fig. 2(*a*), before the contrast agent was injected, the only thing that can be seen is the injector tip inside the jugular vein. The contrast of blood vessels increases from Fig. 2(*b*) to Fig. 2(*h*) after the contrast agent was injected. It shows that only when the contrast agent flows through the blood vessels could the anatomy of blood vessels be clearly observed *in situ*.

Figs. 3(*a*) and 3(*b*) show synchrotron radiation X-ray angiography of the mouse brain blood vessels from the front and from the side, respectively. Because of the limited beam size, the whole brain blood vessels could not be observed at

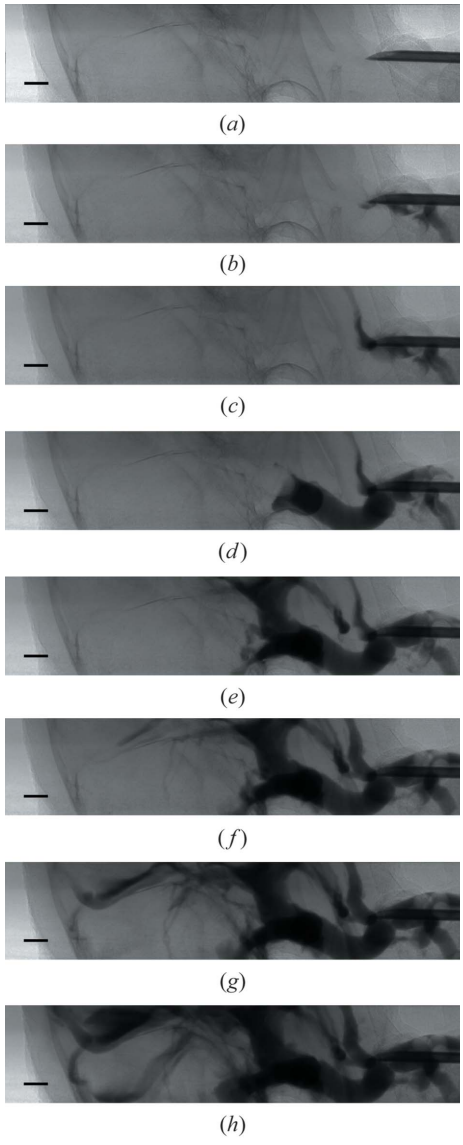

Figure 2
(*a*)–(*h*) Synchrotron radiation X-ray dynamic angiography in the mouse brain using iodine contrast agent. Scale bar: 1 mm.

the same time. The sample position was changed, and a series of images were obtained and patched to form the whole brain image. The network of brain blood vessels could be observed clearly, and the anatomy and diameters of the cerebral arteries were studied, such as the vertebral artery (VA), common carotid artery (CCA), superior cerebral artery (SCA), basilar artery (BA), external carotid artery (ECA), posterior cerebral artery (PCA), internal carotid artery (ICA) and arterior cerebral artery (ACA). The smallest visible vessels were approximately 20–30 µm in diameter [marked by yellow arrows in Fig. 3(*a*, right)].

Fig. 4 shows synchrotron radiation X-ray angiography of the whole-body mouse blood vessels. The main blood vessels in the heart, liver and kidney can be clearly observed. In particular, in the liver and the kidney the small blood vessels branch could be seen clearly.

Using contrast agent, the vascular anatomy from the neck and the thorax to the abdomen could be observed, such as the

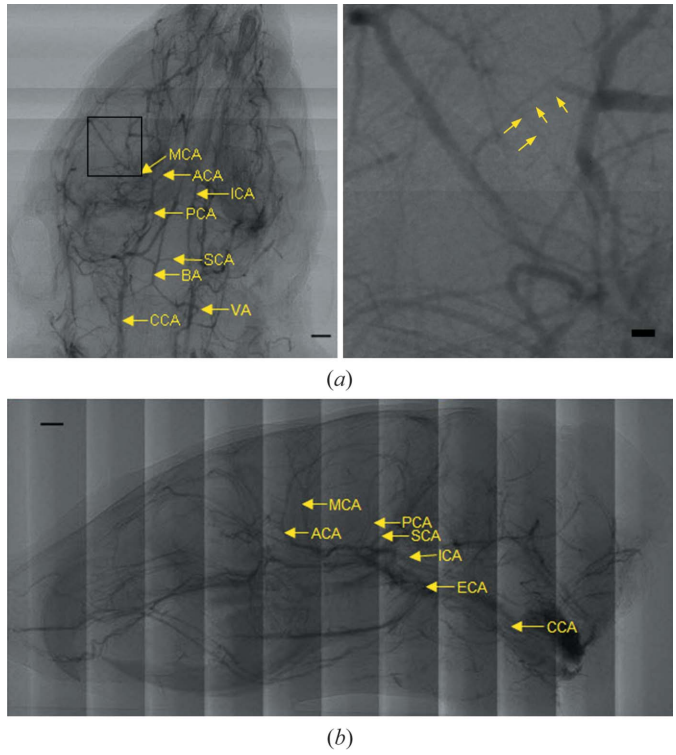

Figure 3
Synchrotron radiation X-ray angiography of a mouse showing the brain blood vessels from the front (*a*) and from the side (*b*). The anatomy of the cerebral arteries in the brain was studied.

left common carotid artery, right common carotid artery, left subclavian artery, brachiocephalic trunk, right subclavian artery, aortic arches, ascending aorta, abdominal aorta, left renal artery, right renal artery, femoral artery, left common iliac artery, right common iliac artery, and so on.

4. Discussion

To obtain morphological and functional biomedical information precisely, a non-invasive imaging technique with high contrast and high-spatial resolution for vascular diseases diagnosis has recently attracted remarkable interest in basic and clinical research. The limitations of the current clinical imaging methods arise mostly from insufficient spatial resolution and contrast. The application of synchrotron radiation in biomedical research is a rapidly growing area, and it has added a new dimension to the use of X-rays in medical imaging.

For the mechanistic study of vascular changes in the early development of brain vascular diseases and tumor angiogenesis, the microangiography system is highly desired for imaging microvessels in the mouse model. However, current angiography systems using a conventional X-ray tube source cannot provide images of arteries less than 200 µm in diameter. In this study, a new synchrotron radiation microangiography system has been developed, which utilizes monochromatic synchrotron radiation as the X-ray source and a high-definition camera or video system as detector. It allows for an enhancement in sensitivity and superior image quality

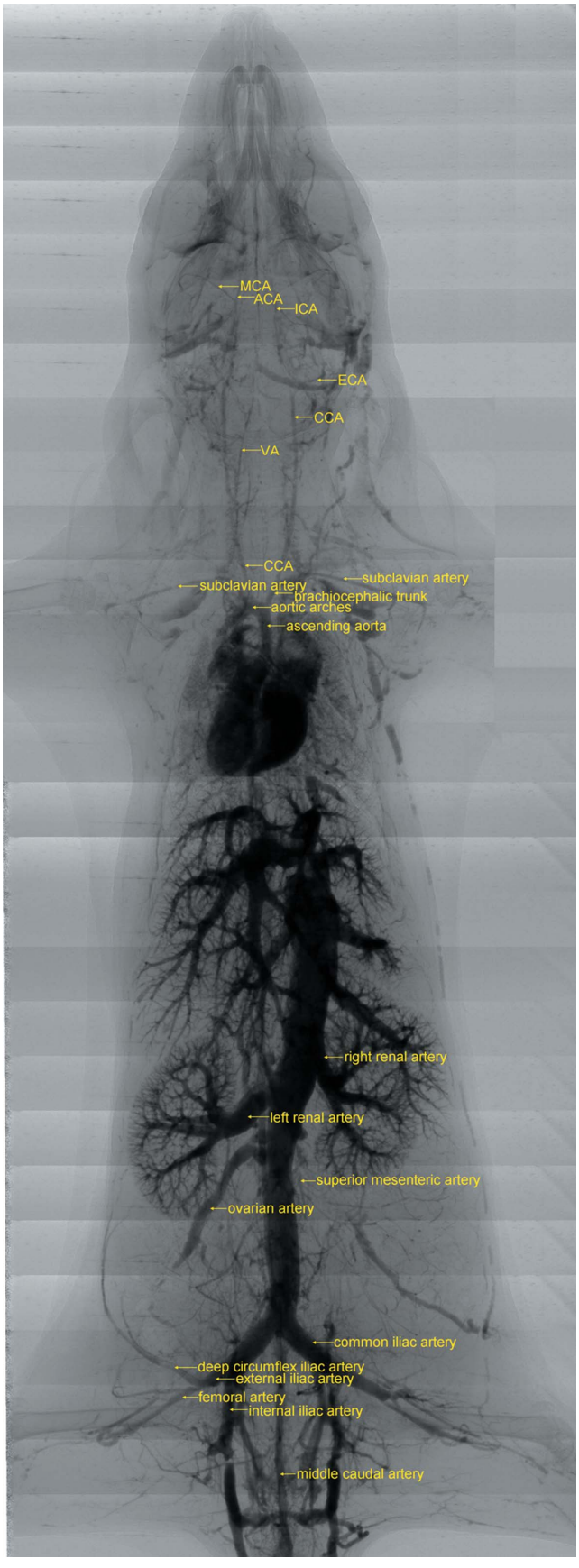

Figure 4
Synchrotron radiation X-ray angiography of a mouse showing the mouse blood vessels in the whole body.

in spatial resolution up to 20 μm. Using the system, we have investigated physiological features of mouse microvasculature in the whole body using synchrotron radiation imaging for the first time, and performed dynamic angiography in the mouse brain with high spatial resolution. The results show that it is feasible to develop novel, dynamic and high-resolution angiography in the whole mouse body using synchrotron radiation.

Despite some limitations, the observations from this study lay the foundation for investigating the potential mechanisms for pathological changes of vascular diseases using the synchrotron radiation microangiography system. Advances in angiographical imaging will accelerate our understanding of the pathogenesis of small vessel disorders disease. Given the low contrast of soft tissues under X-rays, the observation of blood vessels *in vivo* without contrast agents is very difficult. Iodine and barium sulfate were both used as blood vessels contrast agents in this study. However, there lie some problems. Although iodine can be used to image in real time, it is very easy to diffuse from the blood vessel to its surrounding tissue (in minutes), and thus it could not be used to observe blood vessels for a long period of time. Normally, barium sulfate is used only in the digestive tract. It is more stable and not easy to diffuse. However, it could cause serious side effects and mice may die in a short time after the injection.

In addition to new imaging and detection techniques, the development of novel and safe specific targeting contrast agents with a long half-life in circulation is important for both blood vessel structural and functional imaging. Nanoparticle agents for medical imaging have been developed quickly because they have a longer vascular half-life than molecular contrast agents and can be detected by different *in vivo* imaging techniques concurrently (Debbage & Jaschke, 2008; Lucignani, 2009; Howles *et al.*, 2009). Further improvement in the synchrotron radiation microangiography system will enable us to visualize human microvasculature, such as vascular diseases of the brain and tumor in the near future.

This work was performed at beamline BL13W of the SSRF in China, and supported by The National Basic Research Program of China (973 Program 2010CB834303), National Natural Science Foundation of China (50725622, 10705020) and The Science and Technology Commission of Shanghai Municipality (08JC1411900).

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