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Development of Synchrotron Radiation X-ray Intravital Microscopy for *In Vivo* Imaging of Rat Heart Vascular Function

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This study elucidates the vascular internal diameter response of coronary arterial circulation in closed-chest rats to evaluate endothelium-dependent and endothelium-independent vasodilatory ability and to investigate disease mechanisms. For this study, we developed an X-ray intravital microscopy system using a microangiography technique and a synchrotron radiation source at SPring-8. An X-ray direct-conversion type detector with 7-μm spatial resolution was used for real-time imaging. Microangiographic images were stored in a digital frame memory system at a maximum rate of 30 frame/s with a 1024 × 1024-pixel, 10-bit format. In imaging experiments, the small coronary arteries were visualized after iodine contrast agent injection into the coronary artery.

I. Introduction

Intravital microscopy using advanced optical equipment in combination with video technology has been used to investigate disease mechanisms that are reflected by primary manifestation at the level of the micro-circulation unit [1]. Light microscopes can produce sample images with spatial resolution in the sub-micrometer range. However, only a thin layer remains in focus: the entire thickness is out of focus. However, because of its very large depth of field, an X-ray microimaging system using a nearly parallel synchrotron radiation beam presents advantages over light microscopes for clarifying the internal structures of centimeter-sized objects with spatial resolution in the micrometer range.

Imaging research for preclinical *in vivo* small-animal studies has advanced the development of high spatial resolution scanners to reveal fine details in rats, mice, and rabbits since the mid-1990s because drugs can be tested more easily in animals than in humans [2]. Laboratory animal imaging continues to play a decisive role in drug discovery and in elucidating disease mechanisms [3].

A microangiography system with spatial resolution as high as 10 μm was developed at SPring-8 using an X-ray direct-conversion type detector incorporating an X-ray SATICON pickup tube for depiction of tumor-derived angiogenic vessels in a rabbit model of cancer [4]. Since then, the system has been improved with respect to spatial resolution and exposure time (shutter speed) to obtain sequential, sharp, blur-free images of brains in *in vivo* rats and mice [5] and *ex vivo* rat hearts under Langendorff perfusion [6]–[7].

We are planning to undertake radiographical evaluation for the investigation of vascular functions in small coronary arteries by clearly depicting the diameter changes of blood vessels under the influence of a vasoactive agent. For the study, we developed an X-ray intravital microscopy technique using a synchrotron radiation microangiography system. Then, coronary microangiography with spatial resolution of 7 μm and a field of view of 7.0 mm × 7.0 mm was performed for *in vivo* rat heart imaging using a closed chest rat model.

II. Imaging System

Experimental arrangements at the BL28B2 beamline for biomedical imaging using monochromatic synchrotron radiation X-rays are presented in Fig. 1. The beamline consisting of X-ray optical and imaging devices is the experimental station that provides synchrotron radiation users with a high-flux monochromatic X-ray beam for imaging experiments. The source of synchrotron radiation is a storage ring, which uses numerous bending magnets to maintain an 8 GeV electron beam in a closed trajectory. By bending the path of electrons at relativistic speeds, X-rays are emitted at each bending magnet in a direction that is tangential to the beam trajectory. Synchrotron radiation generated by bending magnets produces a fan-shaped and nearly parallel X-ray beam. The single-crystal monochromator selects a single energy of synchrotron radiation. Consequently, X-rays with a small energy bandwidth are used for imaging.

An X-ray imaging system must have a high shutter speed (short exposure time) to produce sharp and blur-free images of objects. For high-speed imaging, we developed a rotating-disk X-ray shutter. The shutter in Fig. 1 was situated between the monochromator and an object. It consists of two disks with radial slots rotating about an axis that is parallel to the X-ray beam. The radial slot width can be changed to adjust the X-ray pulse duration according to the rotation of one disk with another one. The disks also rotate to match timing with the video camera synchronous signals.

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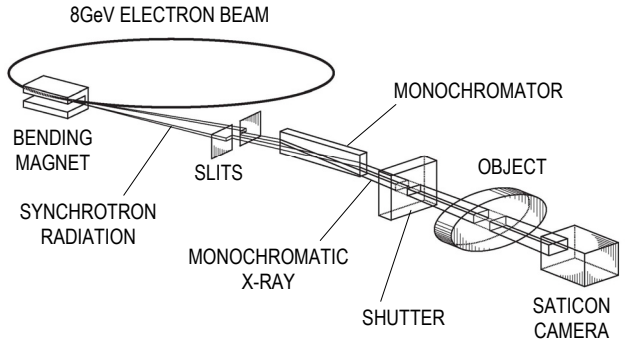

Fig. 1 Experimental arrangement for synchrotron radiation intravital microscopy.

Pulsed monochromatic X-rays transmitted through the object are detected using the X-ray direct-conversion type detector presented in Fig. 2, which incorporates an X-ray SATICON pickup tube. Before the 1990s, video cameras consisted of a pickup tube to capture images. The pickup tube has largely been replaced by a charge-coupled device (CCD). However, some pickup tube cameras are still used for special purpose applications in scientific and industrial fields. For instance, there is no X-ray direct-conversion type CCD that is an alternative to the X-ray direct-conversion type SATICON tube for high-sensitivity and high-spatial-resolution imaging. The tube is a glass cylinder maintained under vacuum; the front end of the tube is a flat plate, the inside of which is coated with a photosensitive material. The X-ray sensitive SATICON tube consists of a photoconductive target layer of amorphous selenium. Absorbed X-rays in the photoconductive layer are converted directly into electron–hole pairs. Then, a charge-density pattern is formed on the photoconductive layer surface. To produce a video signal, a scanning beam of low-velocity electrons reads out the electrostatic image on the surface.

This X-ray SATICON camera for biomedical imaging, which has resolution of 1050 scanning lines, can record images at a maximum rate of 30 frame/s. Sequential images were obtained with the input field of view of 7.0 mm × 7.0 mm. The spatial resolution of the detector is about 7 μm and is similar to the pixel size of 7.0 μm. When synchronizing the timing with the camera's synchronous pulse signals, image signals are converted into a digital format and stored in a frame memory with a format of 1024 × 1024 pixels and 10-bit resolution. An equivalent pixel size projected onto the input window was 7.0 μm for a 1024 × 1024-pixel format.

The distance between the point source in the bending magnet and the detector was about 46 m. A nearly parallel X-ray beam was used for imaging without image blur because of the small X-ray source and the extremely long source-to-object distance. The storage ring was operated at 8 GeV electron beam energy; the beam current was 100 mA. Monochromatic X-ray energy was adjusted to 33.2 keV, which was slightly higher than the iodine K-edge energy to produce the highest contrast image of the iodine contrast agent. The X-ray flux at the object position was ca. 1×10^{10} photon/mm^2/s in imaging experiments.

Fig. 2 Photograph of the X-ray SATICON camera system comprising a camera head (left) and a camera control unit (right).

III. Coronary Microangiography

The selective coronary angiography is the gold standard technique for human studies [8]. In this technique, a catheter tip is placed in the coronary artery and a contrast agent is injected directly into the artery. However, the selective coronary angiography for rat studies is almost impossible because the diameters of the rat coronary arteries are too small for the catheter to be placed in the artery. For this reason, the contrast agent was injected into the aorta close to the origin of the coronary arteries [9] in this study.

After inducing anesthesia with pentobarbital, the rats were intubated for artificial ventilation. A 20-gauge angiocatheter was used to cannulate the right carotid artery to position the tip close to the left descending coronary artery near the aortic valve in the aorta. Arterial pressure was recorded from a catheter inserted into the femoral artery. Iodinized contrast medium (Iomeron 350; Bracco-Eisai Co. Ltd., Tokyo, Japan) delivery was made using a high-speed injector (0.3–0.5 ml bolus 25 ml/s).

Contrast images were recorded from the anterior left ventricle wall during all experiments in the supine position of rats. Using the x-ray shutter trigger, a second set of images was recorded from a conventional camera, which was focused on an oscilloscope display of the pressure signal from the simultaneously recorded left ventricle pressure–volume relations. Inspection of the second set of oscilloscope images was then used to determine the phase of the cardiac cycle for subsequent image analyses.

All animal experiments conformed to the SPring-8 Guide for Care and Use of Laboratory Animals.

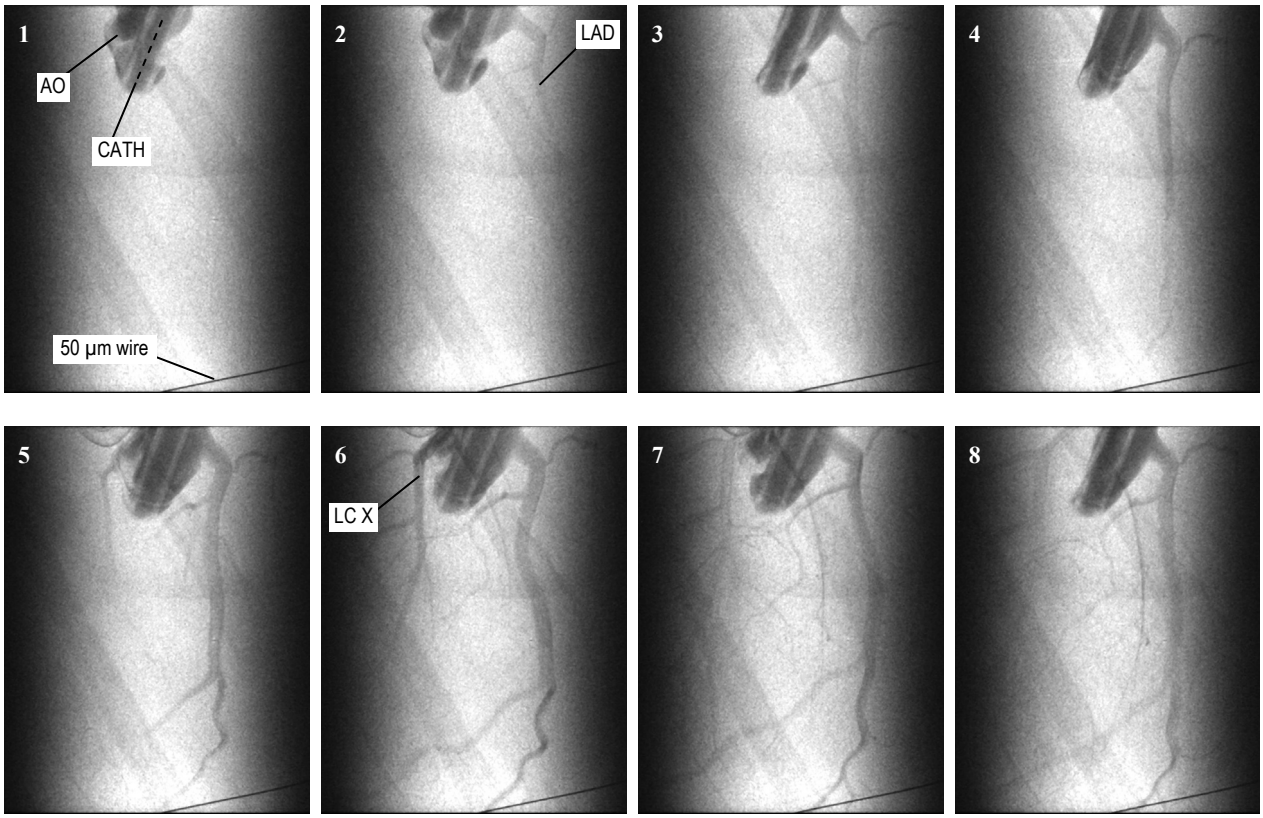

Fig. 3 Microangiographic consecutive images of *in vivo* rat heart. The interval between two consecutive images is 33.3 ms and the exposure time per image was 2.0–2.5 ms. These images show the aorta (AO) close to the aortic valve, a catheter tip (CATH), the left anterior descending coronary artery (LAD), the left circumflex coronary artery (LCX), and a 50-µm tungsten wire.

IV. Results and Discussion

Representative coronary artery consecutive images from a microangiographic sequence are depicted in Fig. 3. The images were rotated 90 deg from the original raw images because the original ones were recorded from the anterior left ventricle wall in the supine position of rats. The image intensities, however, are the same as those of the original ones. These rotated images are comparable with the human coronary angiography. The images were recorded at a rate of 30 frame/s; the interval between two consecutive images is 33.3 ms. The exposure time per image (shutter speed) was adjusted to 2.0–2.5 ms using the X-ray shutter. In each angiographic sequence, 100 images were acquired during the total imaging time of 3.3 s. In Fig. 3, five consecutive images correspond to one heart cycle and a heart rate of about 360 beats/s. This 7.0 mm field of view displays about two-thirds of the entire heart.

The first image in Fig. 3 shows the aorta (AO) and the catheter tip placed near the aortic valve and close to the origin of the left anterior descending coronary artery (LAD). The coronary arteries were visualized after the iodine contrast agent injection into the aorta near the aortic valve. The second–fourth images portray the main trunk of the LAD; the fifth and sixth images depict the main trunk and the small branch arteries of the left circumflex coronary artery (LCX). The fifth–seventh images also show the large and small branch arteries of the LAD. The right coronary artery was not visualized because the catheter tip was placed near the origin of the LAD.

Therefore, we have established the new X-ray intravital microscopy technique using synchrotron radiation for *in vivo* visualization of small coronary arteries. We have also developed the nearly selective coronary angiography technique on injection of the contrast agent into the aorta close to the origin of the coronary artery for the observation of detailed structure and function of small coronary arteries in rats.

Figure 4 presents another coronary artery image depicting the LAD. In this image, a flatten filter operation (Image-Pro Plus; Media Cybernetics, Inc., Maryland, USA) was performed to remove image intensity differences introduced by the non-uniform distribution of the monochromatic X-ray beam, as depicted in Fig. 3. The edge enhancement for the outer boundary of the aorta and the main trunk of the LAD developed secondary to the flatten filter operation. Precise

measurements of blood vessel diameter changes under the influence of a vasoactive agent can be conducted using flattened images. Moreover, third-order branches of coronary arteries are visible in Fig. 3; and small blood vessels of around 50 μm diameter were displayed in the image.

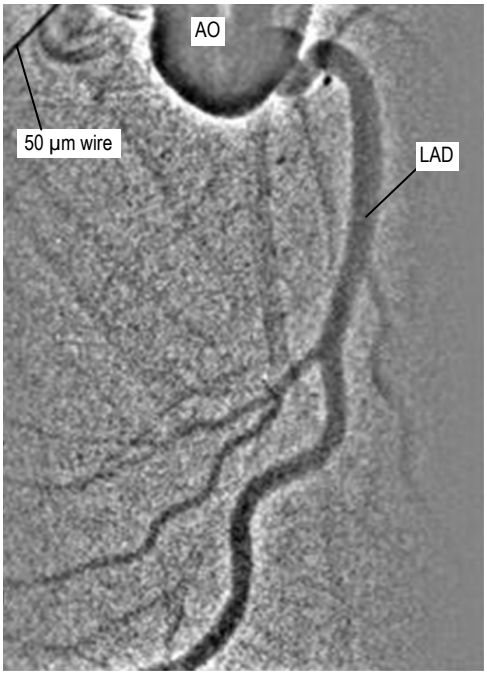

Fig. 4 Microangiographic image of *in vivo* rat heart showing the left anterior descending coronary artery (LAD) and its branches, the aorta (AO), and a 50-μm tungsten wire for vessel size calibration.

In previous *ex vivo* studies using isolated perfused rat hearts [6]–[7], images showed typical diameter changes of the small coronary arteries in response to vasoactive agents and enabled the direct evaluation of vasodilatation caused by acetylcholine, adenosine triphosphate, and sodium nitroprusside stress. In this *in vivo* study using the closed chest rat model, we obtained images of first-order, second-order, and third-order branches of the left anterior descending coronary artery and repeated image recordings were made on the same hearts. Results show that the response magnitude of small arteries/arterioles close to the terminal arterioles, the smallest segments of the arterioles, with diameters of less than 50 μm can also be assessed before and after administration of drugs that modulate endothelial control of the coronary arteries in the *in vivo* closed chest rat model.

V. Conclusion

Laboratory micro-imaging systems using CT, positron emission tomography, magnetic resonance imaging, optical imaging, and ultrasound have neither the spatial nor temporal resolution to detect and measure microvessels accurately in a beating heart. We have established the new X-ray intravital microscopy technique using synchrotron radiation contrast angiography to visualize microvessels in the *in vivo* rat heart following injection of iodine solution into the aorta close to the origin of the coronary artery. The dynamic regulation of vascular tone in the coronary arteries can be determined using *in vivo* coronary microangiography for evaluating vascular endothelial function in healthy and diseased rats.

We are planning to elucidate the vascular internal diameter response of the coronary arterial circulation in closed-chest rats after inducing myocardial infarction to evaluate endothelium-dependent and endothelium-independent vasodilatory ability and to investigate disease mechanisms.

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References

[1] M. D. Menger and H. A. Lehr, "Scope and perspectives of intravital microscopy-bridge over from in vitro and in vivo," *Immunol. Today*, vol. 14, pp. 519–512, 1993.

[2] E. L. Ritman, "Molecular imaging in small animals—roles for micro-CT," *J. Cell Biochem. Supp.,* vol. 39, pp. 116–124, 2002.

[3] L. A. Colby and B. J. Morenko, "Clinical considerations in rodent bioimaging," *Comp. Med.,* vol. 54, pp. 623–630, 2004.

[4] K. Umetani, K. Uesugi, M. Kobatake, A. Yamamoto, T. Yamashita, and Shigeki Imai, "Synchrotron radiation microimaging in rabbit models of cancer for preclinical testing," *Nucl. Instr. and Meth.,* Vol. A609, pp. 38–49, 2009.

[5] K. Kidoguchi, M. Tamaki, T. Mizobe, J. Koyama, T. Kondoh, E. Kohmura, T. Sakurai, K. Yokono, and K. Umetani, "In vivo X-ray angiography in the mouse brain using synchrotron radiation," *Stroke*, vol. 37, pp. 1856–1861, 2006.

[6] H. Iwasaki, K. Fukushima, A. Kawamoto, K. Umetani, A. Oyamada, S. Hayashi, T. Matsumoto, M. Ishikawa, T. Shibata, H. Nishimura, H. Hirai, Y. Mifune, M. Horii, K. Sugimura, S. Suehiro, and T. Asahara, "Synchrotron radiation coronary microangiography for morphometric and physiological evaluation of myocardial neovascularization induced by endothelial progenitor cell transplantation," *Arterioscler. Thromb. Vasc. Biol.,* vol. 27, pp. 1326–1333, 2007.

[7] K. Umetani, K. Fukushima, and K. Sugimura, "Synchrotron radiation microangiography for investigation of metabolic syndrome in rat model," in *Biomedical Engineering*, C. A. B. Mello, Ed. Croatia: IN-Tech, 2009, pp. 107–118.

[8] A. V. G. Bruschke, W. C. Sheldon, E. K. Shirey, and W. L. Proudfit, A Half Century of Selective Coronary Arteriography, *J. Am. Coll. Cardiol.,* vol. 54, pp. 2139-2144, 2009.

[9] S. Paulin, Coronary angiography. A technical, anatomic, and clinical study, *Acta Radiol. Suppl.,* vol. 233, 1964.