Visualization of Penetrating Transmural Arteries In Situ by Monochromatic Synchrotron Radiation

Hidezo Mori, MD; Kazuyuki Hyodo, PhD; Kohsuke Tobita, MD; Mitsuaki Chujo, MSci; Yoshiro Shinozaki, BE; Yasuro Sugishita, MD; Masami Ando, PhD

**Background** Penetrating transmural arteries with a diameter of <500 μm are considered to be a critical vascular component that causes a transmural variation of myocardial blood flow under various pathophysiological conditions. However, the conventional coronary angiographic system is not oriented to the visualization of such small arteries as these.

**Methods and Results** We magnified and monochromatized the inherently narrow beam (3 mm along the vertical direction) of synchrotron radiation by using an asymmetrically cut silicon crystal with 311 reflecting planes to obtain a monochromatic x-ray with a relatively large beam size (60×25 mm) and with an energy of just above (+130 eV) the K-absorption edge of the contrast materials (33.17 and 37.41 keV for iodine and barium, respectively). We irradiated dogs or excised hearts with the monochromatic x-ray and obtained coronary angiograms using an image intensifier and video system with a spatial resolution of 170 μm. In the anesthetized dog experiments, we visualized the transmural penetrating arteries (5 to 15 mm in length) arising every 4 to 7 mm from the epicardial branch. Visualization of these arteries filled with heavy element-loaded microspheres (15 μm in diameter) in the excised-heart experiments, in which the monochromatic x-ray was irradiated to the hearts through a 10- to 20-cm acrylic plate, indicated that this system could be used for human patients, in whom body absorption of x-ray is substantial.

**Conclusions** Coronary angiogram by means of monochromatic x-ray is useful for a precise evaluation of coronary circulation, both in clinical settings and in physiological animal experiments. (*Circulation. 1994;89:863-871.*)

**Key Words** • arteries • angiography • microspheres

In the coronary circulatory system, there is a transmural variation in susceptibility to myocardial ischemia under various pathophysiological conditions. Penetrating transmural arteries with a diameter of <500 μm that traverse from the epicardium to endocardium are considered a critical vascular component that causes the transmural variations in blood flow. However, conventional coronary angiography is not oriented to the evaluation of penetrating transmural arteries. The spatial resolution of the x-ray angiographic system depends on x-ray source (the photon density and the energy level) and the resolution of the detector (image intensifier and video system). Although the spatial resolution of the image intensifier and video system in conventional coronary angiographic systems is about 170 μm (3 line pairs per millimeter), the overall resolution is not sufficient for evaluating coronary arterial branches with a diameter of <500 μm. This is due mainly to the insufficient x-ray source. We describe a new coronary angiographic system for visualizing penetrating transmural arteries and possibly even smaller branches in the near future. With our system, we can detect small amounts of contrast materials in small vessels by applying monochromatic synchrotron radiation as an x-ray source. The synchrotron radiation is characterized by high brilliance, tunability of the energy level, and extreme collimation. The high brilliance allows us to increase the sensitivity to detect small amounts of contrast material in a short irradiation period, and the tunability of x-ray energy allows us to obtain the maximum difference for the x-ray absorption coefficient between the contrast material and the body tissue.

**Methods**

These studies were performed in the period of December 1991 to March 1993 at the National Laboratory for High Energy Physics in Tsukuba, Japan. Synchrotron radiation was derived from an accumulation ring (AR) of electrons with an accelerated energy of 6.5 GeV and an average beam current of 25 mA at beamline AR-NES. The synchrotron radiation beam via this beamline, AR-NES, is illuminated by a bending magnet and has a divergence angle of 10 mrad along a horizontal plane.

**Description of the Imaging System**

The experimental setup is illustrated in Fig 1, and the parameters of the x-ray source and detecting system are summarized in Table 1 (original view). In this setup, the inherently narrow beam (3 mm along the vertical direction) of synchrotron radiation with a photon density of 8×10^9 photons/mm^2 per second was monochromatized at an optimal energy level and magnified eight times (from 3 to 25 mm) by means of an asymmetrically cut silicon crystal with 311 reflecting planes in front of the objects (Fig 1). By irradiating x-ray of continu-
ous energy bandwidth to a silicon crystal with a lattice plane, the X-ray is monochromatized at a certain energy (Bragg reflection). The angle of the coincidental beam direction and the lattice plane of the silicon crystal (Bragg angle, \( \theta \)) determine the energy of the diffracted X-ray. By applying an asymmetrically cut crystal, we can monochromatize and magnify the synchrotron radiation beam simultaneously. The magnification ratio (M) is determined by the angle (\( \alpha \)) of the lattice plane and the surface of the crystal and the Bragg angle [\( M = \frac{\sin(\theta + \alpha)}{\sin(\theta - \alpha)} \)]. The estimated photon density in front of an object is \( 1 \times 10^7 \) photons/mm\(^2\) per second (Table 1) in the present setup. The beam size in front of an object (radius field) is 60×25 mm. We used an image intensifier (RTP 9211G with 7-in mode, Toshiba, Tokyo) as an X-ray detector. The images formed on the fluorescent screen of the image intensifier were sent to a CCD video camera (XC77, Sony, Tokyo) and stored on videocassette (KCA60K, Sony, Tokyo) by means of a videocassette recorder (VBO-7600, Sony, Tokyo) or on digital radiographic film (Imaging Plate, Fuji Film, Tokyo). The spatial resolution of the image intensifier and video system was 170 \( \mu \)m (3 line pairs per millimeter). This setup is exactly the same as that for the transvenous coronary angiography with synchrotron radiation by the National Laboratory for High Energy Physics in Tsukuba.\(^5\)

Visualization of Penetrating Transmural Arteries In Situ

We visualized penetrating transmural arteries arising from diagonal branches in three mongrel dogs (weight, 14 to 18 kg) by means of a monochromatic synchrotron radiation system. We introduced anesthesia, controlled ventilation, and performed left thoracotomy and pericardiotomy. We then dissected the left anterior descending artery between the first and second diagonal branches and the left subclavian artery. We placed a bypass circuit made of silicon tubing between the arteries and monitored coronary arterial flow using an electromagnetic flow meter (MBF 2100, Nihon Kohden, Tokyo), the probe of which was set in the bypass. We temporarily ligated the distal portion of the left anterior descending artery and infused 3 to 5 mL of nonionic iodinated contrast media (Iopamilon, Nihon Schering Co Ltd, Osaka) with 37% (wt/wt) iodine into the bypass circuit via a three-way stopcock by means of an autoinjector (Nemoto Kyorindo Corp, Tokyo) while irradiating the dog with monochromatic synchrotron radiation.

Table 1. Descriptions of the Parameters for the Original View and for the Magnified View in the Near Future

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Original View</th>
<th>Magnified View</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incident beam size, mm</td>
<td>60×3</td>
<td>60×3</td>
</tr>
<tr>
<td>First crystal</td>
<td>Si (311) ( \alpha = 5^\circ )</td>
<td>Si (311) ( \alpha = 3^\circ ) or ( 5^\circ )</td>
</tr>
<tr>
<td>Energy level of monochromatic X-ray, keV</td>
<td>33.30</td>
<td>33.30</td>
</tr>
<tr>
<td>FWHM, eV</td>
<td>130</td>
<td>130</td>
</tr>
<tr>
<td>Radiation field, mm</td>
<td>60×25</td>
<td>10×9-10</td>
</tr>
<tr>
<td>Estimated photon density at an object, photons/mm(^2) per second</td>
<td>( 1 \times 10^9 )</td>
<td>1.3×10(^9)</td>
</tr>
<tr>
<td>Second crystal</td>
<td>None</td>
<td>Si (311) ( \alpha = 3^\circ ) or ( 5^\circ )</td>
</tr>
<tr>
<td>Third crystal</td>
<td>None</td>
<td>Si (311) ( \alpha = 3^\circ ) or ( 5^\circ )</td>
</tr>
<tr>
<td>Spatial resolution, ( \mu m )</td>
<td>170</td>
<td>60-20</td>
</tr>
</tbody>
</table>

The parameters of the present imaging system (original view) and the new system in the near future (magnified view) are comparatively described. The original view is designed to visualize penetrating transmural arteries with a spatial resolution of 170 \( \mu m \). The magnified view is designed to evaluate precisely the change in the vessel diameters and to visualize smaller arteries with a spatial resolution of 20 to 60 \( \mu m \).

Si indicates silicon crystal; \( \alpha \), the angle of the lattice plane and the surface of the crystal; and FWHM, full width at half maximum of the monochromatic X-ray spectra with a peak energy level of 33.30 keV.
radiation. The energy level of synchrotron radiation was set at just above (+130 eV) the K-absorption edge for iodine (33.17 keV). In these experiments, we took coronary angiograms at baseline, reduced coronary perfusion pressure, coronary vasodilation with adenosine (5 µg/kg per minute, IC administration), and/or coronary vasoconstriction with endothelin (40 pmol, IC administration).

Visualization of Penetrating Transmural Arteries in the Excised Hearts

In three excised-heart experiments, we confirmed the optimal energy level of monochromatic synchrotron radiation needed for visualizing small coronary arteries and evaluated whether the present system could visualize small coronary arteries in patients as well as in dogs. We anesthetized three dogs with a weight of 8 to 12 kg by intravenous injection of pentobarbital (30 mg/kg) and controlled ventilation by an artificial respirator via an endotracheal tube. After the left thoracotomy and pericardiectomy, we dissected the left subclavian artery and the left anterior descending artery (two dogs) or the left circumflex artery (one dog) at the proximal portion and set a bypass between the two arteries. We injected 1 to 2 x 10⁷ of barium-loaded polystyrene microspheres with a mean diameter of 15 µm and with a barium concentration of 29.4% into the bypass toward the left anterior descending artery in one of the three dogs and the same amount of iodine-loaded (37% concentration) acrylic microspheres in the remaining two dogs. By use of these procedures, the small coronary arteries were filled with the radiopaque microspheres retrogradely from precapillary coronary arteries with a diameter of about 15 µm. We then killed the dogs by injecting a large amount of pentobarbital intravenously, and we excised and fixed the hearts in 10% formalin solution for several days. We performed coronary angiography on these excised hearts by using monochromatic synchrotron radiation with an energy of above (+130 eV) and below (~130 eV) the K-absorption edge for barium (37.41 keV) or for iodine (33.17 keV). To simulate attenuation of x-ray by the human body, monochromatic synchrotron radiation was irradiated to the canine hearts through an acrylic plate 20 cm thick in the heart treated with the barium-loaded microspheres and through a 10-cm acrylic plate in the two hearts with the iodine-loaded microspheres. In general, the degree of attenuation of irradiated x-ray by tissue depends on the mass attenuation coefficient for tissue and the body thickness \( t = \text{L}_a x \mu \text{m} \) where \( \text{L}_a \) and \( \mu \) indicate thickness of x-ray behind and in front of the object, respectively; \( \mu \) indicates mass attenuation coefficient for the tissue; and \( t \) indicates thickness of the tissue); the photon density reduces to approximately 1/1000 through human body with a thoracic thickness of 20 cm.

All the animal experiments were performed in accordance with the guidelines on animal use of Tokai University School of Medicine, which conform to the guiding principles of the American Physiological Society.

Results

Visualization of Penetrating Transmural Arteries In Situ

This coronary angiography visualized transmural penetrating arteries originating every 4 to 7 mm from the diagonal branches in situ (Fig 2, top). We identified two groups of penetrating transmural arteries: longer ones of 10 mm or more, which probably supplied the endocardial layers (the larger arrow without bar), and shorter ones of approximately 5 mm or less, which probably supplied the epicardial or middle layers (the smaller arrow without bar). Endothelin administration in the same dog reduced the coronary blood flow to approximately 25% of the baseline flow. Under this condition, narrowing of the penetrating transmural arteries and the diagonal branch became obvious (Fig 2, bottom). We compared the angiograms taken during a reduced coronary perfusion pressure and during vasodilation with adenosine in the other dog (Fig 3). We could identify lightly stained penetrating transmural arteries with a long washout time of the contrast materials under reduced coronary perfusion (blood flow of the anterior descending artery of 6 mL/min, <20% of the baseline, Fig 3, top). Under adenosine treatment (coronary blood flow of 60 mL/min, 188% of the baseline), we identified densely stained penetrating transmural arteries and tissue (Fig 3, bottom).

Visualization of Penetrating Transmural Arteries in the Excised Hearts

The coronary angiograms of the excised hearts demonstrated the advantage of tuning the synchrotron radiation energy and the possibility of applying the present method to clinical settings. The monochromatic x-ray irradiation with an energy level of just above (+130 eV) the K-absorption edge of barium (37.41 keV) visualized the small coronary arteries penetrating the heart wall perpendicularly from the anterior epicardial surface to the heart cavity (penetrating transmural arteries, Fig 4, top). In contrast, the arteries are poorly visualized just below the K-absorption edge (~130 eV, Fig 4, bottom). These observations confirmed the advantage of tunability of x-ray energy in the synchrotron radiation system. As shown in Fig 5, we could identify the penetrating transmural arteries of the left anterior descending artery as well as of the left circumflex artery, filled with iodine-loaded microspheres, by using monochromatic synchrotron radiation with an energy just above the K-absorption edge of the iodine. The copper wire with a diameter of 150 µm shown by the arrow in Fig 5 indicated that the present system could visualize small arteries with a diameter of approximately 150 µm. The monochromatic x-ray was irradiated to the dog after passing through the 20-cm-thick acrylic plate used in the experiments shown in Fig 4 and through the 10-cm plate in the experiments in Fig 5. These observations indicated that the present system can maintain enough photon density in front of the detector even in humans, since the degrees of attenuation of x-ray intensity in these experiments were designed to equal those in human beings with a 10- and 20-cm thoracic thickness, respectively.

Discussion

New Observations From This Study

The present study demonstrates that a coronary angiographic system using monochromatic synchrotron radiation can visualize transmural penetrating coronary arteries in anesthetized dogs (Figs 2 and 3), and that this system can very likely be applied to clinical settings (Figs 4 and 5). Possible clinical benefits of the visualization of penetrating transmural arteries are detection of organic and/or functional abnormalities in these small arteries in atherosclerotic heart disease, the so-called syndrome X, severe aortic valvular disease, hy-
Coronary angiograms of beating heart in an anesthetized dog. Top, Angiogram taken at baseline condition without any pharmacological intervention (blood flow of left anterior descending artery of 27 mL/min). Bottom, Angiogram taken after endothelin treatment (40 pmol IC). The energy of the monochromatic synchrotron radiation was set at just above (+130 eV) the K-absorption edge of iodine (33.17 keV). The larger and smaller arrows with bars at top indicate the tip of the bypass with an internal diameter of 2 mm and the diagonal branch of the left anterior descending artery, respectively. The larger and smaller arrows without bars indicate a long and a short penetrating transmural artery, which probably feed deep myocardial layer and superficial layer, respectively.

Pertrophic cardiomyopathy, diabetic heart disease, transplanted hearts suffering rejection, and others. Furthermore, the system can be applied to a clinical evaluation of the small arteries in other organs.

The radiation doses were not obviously different between the present synchrotron radiation system and the conventional angiographic systems. The surface doses of the monochromatic synchrotron radiation system described, a conventional coronary angiographic system, and a digital subtraction angiographic system are approximately 1.6, 1.3, and 1.0 R/s (16, 13, and 10 mSv in dose equivalence), respectively. These doses allow us to obtain sufficient signal-to-background ratio of approximately 300 to 1000 at each pixel of the detecting systems, assuming that the x-ray is attenuated to 1/1000 by the human body.
In contrast, the photon numbers at critical energy level (just above the K-absorption edge of iodine of 33.17 keV) are quite different between these systems. The exposure of the photons with the critical energy level produces the biggest difference in x-ray absorptions because of iodine and because of tissue as shown by their mass attenuation coefficients in Fig 6. Because the monochromatic synchrotron radiation with peak energy of 33.30 keV has a quite small full width at half maximum, 130 eV (Table 1), most of the photons are included in the critical range. In contrast, the fractions of the photons with the critical energy level in the x-ray sources of the conventional angiographic systems with a continuous energy band are much smaller than the monochromatic synchrotron radiation. These considerations explain the marked difference in the ability to detect small coronary arterial branches filled with a small amount of contrast materials and the lack of marked difference in their radiation doses between the monochromatic synchrotron radiation system and the conventional angiographic systems.

Comparison of the Current System to the Intravenous Coronary Angiographic Systems Using Monochromatic Synchrotron Radiation

The research group from the Stanford Synchrotron Radiation Laboratory, Calif, initially applied monochromatic synchrotron radiation to clinically oriented intravenous coronary angiography; the National Laboratory for High Energy Physics in Tsukuba, Japan, as well as German (DESY, Hamburg, Germany) and Russian research groups (Nobosibirsk, Russia) followed the Stanford group. The differences of these systems are summarized in Table 2. The Stanford group as well as the German and Russian groups developed a dual-beam and dual-detector system to record high-energy (above K edge) and low-energy (below K edge) images of the major coronary arterial branches, and the

Fig 3. Coronary angiograms under reduced coronary perfusion pressure (top) and under intracoronary adenosine treatment (bottom).
Stanford and German groups produced subtraction images of these (K-edge subtraction) in several human subjects. The K-edge subtraction was able to improve the quality of the images by excluding artifacts arising from temporal subtraction. Since their system was a one-dimensional scanned imaging system, moving images of the coronary arterial branches were not available. The group from the National Laboratory for High Energy Physics in Tsukuba, Japan, has been working on moving images of coronary arterial trees in real time with intravenously injected contrast materials,3-5 and we used the same experimental setup in the present microcoronary angiographic project as for the intravenous coronary angiogram. To obtain two-dimensional images in our system, the inherently collimated beam of synchrotron radiation had to be magnified in front of the object by means of an asymmetrically cut silicon crystal. The magnification is accompanied by a partial loss of photon flux density in front of the object. Therefore, this leads to a question as to whether the present angiographic system at beamline AR-NE5 has sufficient sensitivity to visualize a coronary artery in humans in which a significant attenuation of photon density through the tissue (approximately 1/1000) has been suspected. The results from the excised-heart experiments (Figs 4 and 5) indicated that the present microcoronary angiographic system could visualize the small coronary arteries under conditions of x-ray attenuation similar to humans with a thoracic thickness of 10 to 20 cm. In the present microcoronary angiographic system, contrast materials are injected superselectively into a local coronary arterial branch; therefore, the concentration of contrast materials in the target arteries is much higher than under intravenous coronary angiography. This gives an advantage in obtaining good contrast of small coronary arteries with the present microcoronary angiographic system as opposed to the intravenous coronary angiographic system.

Modifications of the Current Coronary Angiographic System in the Near Future

We are trying the following modifications of the present angiographic system to obtain better contrast images of small coronary arteries. First, we are changing...
the arrangement of the silicon crystal for monochromatization, as summarized in Table 1, to obtain a magnified view. In this setup, the synchrotron radiation is monochromatized by use of a silicon crystal with an asymmetrically cut surface in front of the object, is irradiated to the object, and is magnified along the vertical axis by a second silicon crystal and magnified again along the horizontal axis by a third silicon crystal between the object and the image intensifier. This magnified view will allow us to quantify the diameters of the penetrating transmural arteries more exactly and visualize even smaller coronary arteries arising from the penetrating transmural arteries. The photon density at the object will become 1 to $3 \times 10^9$ photons/mm$^2$ per second, and the spatial resolution of the detecting system will be improved to 20 to 60 μm. However, the radiation field in this setup will become smaller (10×9 to 10 mm). An alternative method to improve the spatial resolution is to apply a more precise detector. In the present system, the number of CCD pixels of the video system was 512×512. By using a video system with a pixel number of 1024×1024 together with the relocation of the asymmetrical silicon crystals, the spatial resolution might be further improved down to 15 μm. By applying a focused beam of synchrotron radiation and using a CCD video camera system with a charge coupled device, we might be able to quantitatively evaluate even contrast images of capillary vessels in situ. However, a finer detecting device is usually associated with a smaller visual field. Therefore, the beating of the heart would make it difficult to keep the small vessels in the small visual field of the detecting system.

Applying the K-edge subtraction method is another modification of our system to improve the quality of the images. Rubenstein et al$^{17,18}$ described a system using two beams of synchrotron radiation with different energies for K-edge subtraction. Nishimura et al$^{14}$ and Fukagawa et al$^{15}$ reported the possibility of K-edge subtraction with a single synchrotron radiation beam by changing the energy within 2 milliseconds and detecting the images with different energies using two sets of video cameras. Umetani et al$^{16}$ demonstrated moving images of the canine coronary artery with a single synchrotron

![Figure 5](http://circ.ahajournals.org/)

**Fig 5.** Coronary angiograms of excised canine heart of which small coronary arteries arising from the left anterior descending artery were filled with iodine-loaded acrylic microspheres 15 μm in diameter. The arrow indicates a copper wire with a diameter of 150 μm. This angiogram was taken by monochromatic synchrotron radiation with an energy of just above (+130 eV) the K-absorption edge of iodine (33.17 keV).

![Figure 6](http://circ.ahajournals.org/)

**Fig 6.** Plot of relations of photon energy level (x axis) and mass attenuation coefficients (y axis) are shown. The mass attenuation coefficient for iodine is discrete at 33.17 keV (K-absorption edge for iodine), and the biggest difference in the mass attenuation coefficients for iodine and bone or muscle was noted just above the K-edge.
Use of Synchrotron Radiation in the Medical Field Now and in the Future

Synchrotron radiation has certain potential in medical fields. X-ray diffraction with synchrotron radiation is quite useful for the analysis of fine molecular structure.\(^\text{17}\) X-ray microscopy using synchrotron radiation has great potential for dynamic observation of biological specimens.\(^\text{18}\) X-ray fluorescence spectrometry using monochromatic synchrotron radiation increases the ability to analyze nonradioactive tracer elements in tissue,\(^\text{19}\) and Mori et al.\(^\text{6}\) have already reported a method using monochromatic synchrotron radiation to measure regional blood flow with nonradioactive microspheres.

Synchrotron radiation has thus far been available only in a limited number of institutes. It was initially seen, for large-scale projects in modern physics, as an undesirable loss of energy from accelerated positrons or electrons. The facility required for the generation of synchrotron radiation itself is much smaller than that for large-scale projects in physics,\(^\text{20,21}\) and the use of the synchrotron radiation from a small ring has been expanded in the scientific and industrial fields. Application of synchrotron radiation in the medical field has been delayed compared with other scientific or industrial fields. However, certain Japanese heavy industrial companies are proposing practical plans for a synchrotron radiation facility for medical applications.\(^\text{22}\) According to their report, the ring for acceleration and accumulation of positrons or electrons, from which we will be able to obtain hard x-rays with an energy level of more than 35 keV, should have a diameter of 13 m. They estimate that the cost for the facility described above would be $30 million or more. Major maintenance costs include electricity and liquid helium for cooling the superconductive magnet. However, increased demand for the synchrotron radiation facility in the medical field might reduce the cost of the facility; such demand will depend also on exploratory studies in the medical field with synchrotron radiation in the near future.

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References


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