

Enhanced real-time magnification angiography utilizing a 100- μ m-focus x-ray generator in conjunction with an image intensifier

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ABSTRACT

A microfocuss x-ray tube is useful in order to perform magnification digital radiography including phase-contrast effect. The 100- μ m-focus x-ray generator consists of a main controller for regulating the tube voltage and current and a tube unit, with a high-voltage circuit and a fixed anode x-ray tube. The maximum tube voltage, current, and electric power were 105 kV, 0.5 mA, and 50 W, respectively. Using a 3.0-mm-thick aluminum filter, the x-ray intensity was 26.0 μ Gy/s at 1.0 m from the source with a tube voltage of 60 kV and a current of 0.50 mA. Because the peak photon energy was approximately 35 keV using the filter with a tube voltage of 60 kV, the bremsstrahlung x-rays were absorbed effectively by iodine-based contrast media with an iodine K-edge of 33.2 keV. Real-time magnification radiography was performed by twofold magnification imaging with an image intensifier camera, and angiography was achieved with iodine-based microspheres 15 μ m in diameter. In angiography of non-living animals, we observed fine blood vessels of approximately 100 μ m with high contrasts.

Keywords: real-time magnification radiography, magnification angiography, 100- μ m-focus tube, tungsten target, image intensifier, phase-contrast effect

1. INTRODUCTION

To perform high-speed biomedical radiography, several various flash x-ray generators using cold-cathode tubes have

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been developed.¹⁻⁴ In particular, quasi-monochromatic flash x-ray generators⁵⁻¹⁰ have been designed to perform preliminary experiments for producing clean K-series x-rays, and higher-harmonic hard x-rays have been observed in a weakly ionized linear plasma of copper and nickel. However, in monochromatic flash radiography, difficulties in increasing x-ray duration and in performing x-ray computed tomography (CT) have been encountered. In view of this situation, we have developed steady-state characteristic x-ray generators to produce clean characteristic x-rays, since bremsstrahlung rays are not emitted in the opposite direction to that of electron trajectory.

Monochromatic parallel beams produced from a synchrotron using silicon crystals have been employed in phase-contrast radiography^{11,12} and enhanced K-edge angiography.^{13,14} In particular, the parallel beams with photon energies of approximately 35 keV have been employed to perform iodine K-edge angiography, because the beams are absorbed effectively by iodine-based contrast media with a K-absorption edge of 33.2 keV.

Without using synchrotrons, phase-contrast radiography for edge enhancement can be performed using a microfocus x-ray tube, and the magnification radiography including the phase-contrast effect¹⁵ has been applied in mammography achieved with a computed radiography (CR) system¹⁶ (Regius 190, Konica Minolta) with a sampling pitch of 43.8 μm using a 100- μm -focus molybdenum tube. Subsequently, we have developed a cerium x-ray generator¹⁷⁻¹⁹ to perform enhanced K-edge angiography using cone beams, and have succeeded in observing fine blood vessels and coronary arteries with high contrasts using cerium K α rays of 34.6 keV. However, it is difficult to design a small focus cerium tube for angiography.

Magnification radiography is useful in order to improve the spatial resolution in digital radiography, and narrow photon energy bremsstrahlung x-rays with a peak energy of approximately 35 keV from a microfocus tungsten tube are useful to perform high-contrast high-resolution angiography. In magnification radiography, scattering beams from radiographic objects can be reduced without using a grid.

In this research, we employed a 100- μm -focus tungsten tube, used to perform real-time magnification radiography, including angiography, using an image intensifier (II) in conjunction with a CCD camera.

2. X-RAY GENERATOR

Figure 1 shows the block diagram of a microfocus x-ray generator used in this experiment, and the generator consists of a main controller, an x-ray tube unit with a Cockcroft-Walton circuit, an insulation transformer, and a 100- μm -focus x-ray tube. The tube voltage, the current, and the exposure time can be controlled by the controller. The main circuit for producing x-rays employs the Cockcroft-Walton circuit in order to decrease the dimensions of the tube unit. In the x-ray tube, the positive and negative high voltages are applied to the anode and cathode electrodes, respectively. The filament heating current is supplied by an AC power supply in the controller in conjunction with an insulation transformer which is used for isolation from the high voltage from the Cockcroft-Walton circuit. In this experiment, the tube voltage applied was from 45 to 70 kV, and the tube current was regulated to within 0.50 mA (maximum current) by the filament temperature. The exposure time is controlled in order to obtain optimum x-ray intensity, and narrow-photon-energy bremsstrahlung x-rays are produced using a 3.0-mm-thick aluminum filter for absorbing soft x-rays.

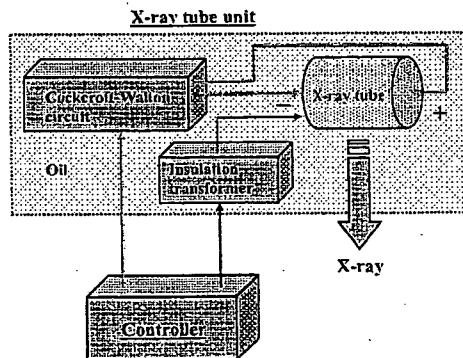


Fig. 1. Block diagram of the x-ray generator.

3. RESULTS AND DISCUSSION

3.1 X-ray intensity

The x-ray intensity was measured by a Victoreen 660 ionization chamber at 1.0 m from the x-ray source using the filter (Fig. 2). At a constant tube current of 0.50 mA, the x-ray intensity increased when the tube voltage was increased. At a tube voltage of 60 kV, the intensity with the filter was 26.0 $\mu\text{Gy/s}$.

3.2 X-ray Spectra

In order to measure x-ray spectra, we employed a cadmium telluride detector (XR-100T, Amptek) (Fig. 3). When the tube voltage was increased, the bremsstrahlung x-ray intensity increased, and both the maximum photon energy and the spectrum peak energy increased.

In order to perform K-edge angiography, bremsstrahlung x-rays of approximately 35 keV are useful, and the high-energy bremsstrahlung x-rays decrease the image contrast. Using this filter, because bremsstrahlung x-rays with energies higher than 60 keV were not absorbed easily, the tube voltage for angiography was determined as 60 kV by considering the filtering effect of radiographic objects.

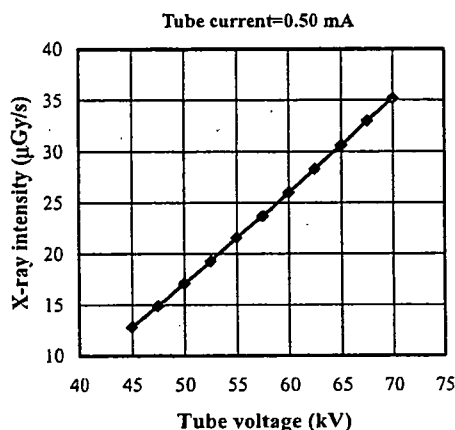


Fig. 2. X-ray intensity ($\mu\text{Gy/s}$) as a function of tube voltage (kV) with a tube current of 0.50 mA.

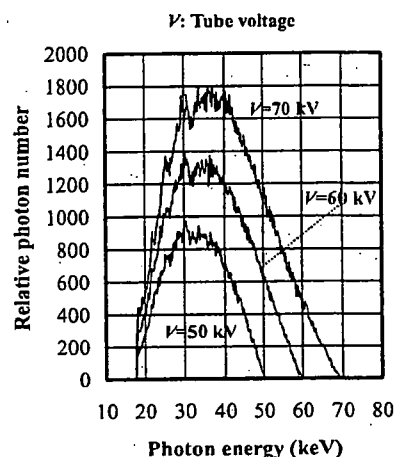


Fig. 3. Bremsstrahlung x-ray spectra measured using a cadmium telluride detector with changes in the tube voltage.

3.3 Magnification radiography

The magnification radiography was performed by twofold magnification imaging using the II camera and the filter at a tube voltage of 60 kV, and the distance between the x-ray source and the II was 1.0 m (Figs. 4 and 5). First, the spatial resolution of magnification radiography was made using a lead test chart (Fig. 6). In the magnification radiography, 109 μm lines (4.6 line pairs/mm) were visible. Subsequently, radiography of tungsten wires coiled around rods made of polymethyl methacrylate (PMMA) was performed (Fig. 7). Although the image contrast decreased somewhat with decreases in the wire diameter, a 50- μm -diameter wire could be observed.

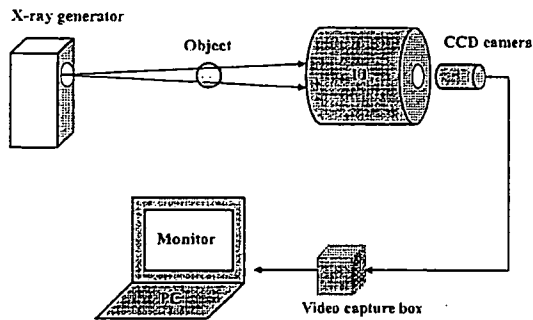


Fig. 4. Real-time magnification imaging using an image intensifier camera (low-resolution mode) in conjunction with a microfocus tube.

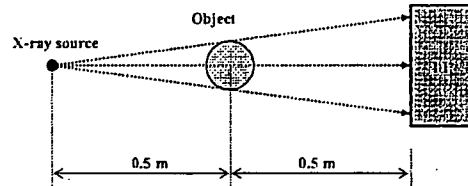


Fig. 5. Twofold magnification imaging.

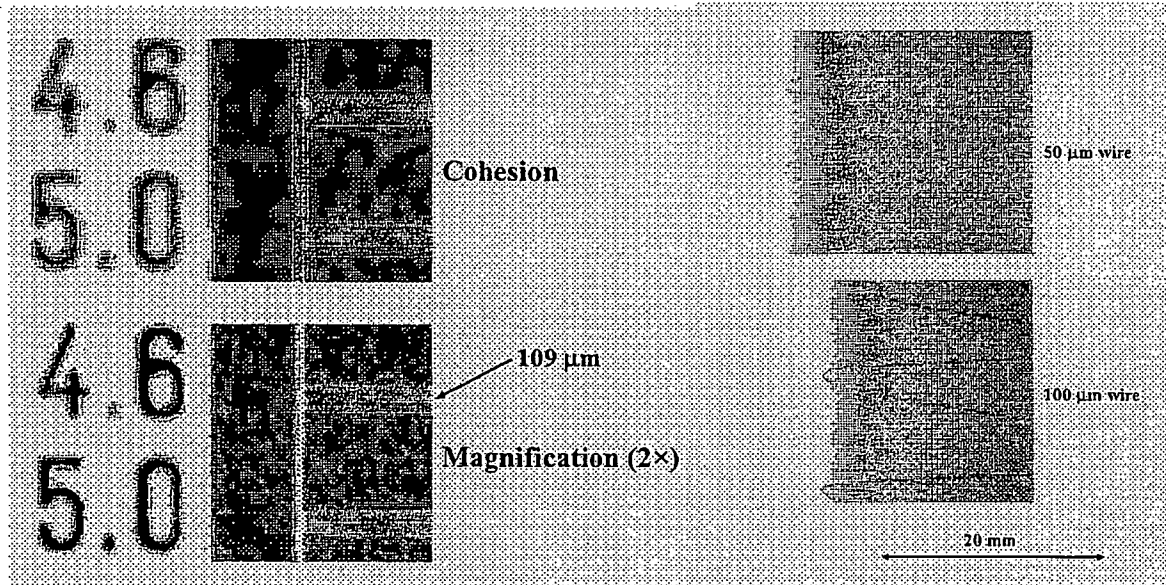


Fig. 6. Radiograms of a test chart for measuring the spatial resolution.

Fig. 7. Radiograms of tungsten wires coiled around PMMA rods.

3.4 Enhanced magnification angiography

Figure 8 shows the mass attenuation coefficients of iodine at the selected energies; the coefficient curve is discontinuous at the iodine K-edge. The effective bremsstrahlung x-ray spectra for K-edge angiography are shown above the iodine K-edge. Because iodine contrast media with a K-absorption edge of 33.2 keV absorb the rays easily, blood vessels were observed with high contrasts.

The magnification angiography was performed at the same conditions using iodine microspheres of 15 μm in diameter, and the microspheres (containing 37% iodine by weight) are very useful for making phantoms of non-living animals used for angiography. Angiograms of a rabbit heart on the turn table is shown in Fig. 9, and the coronary arteries are visible. Figure 10 shows angiograms of a dog heart in an xy table, and blood vessels of approximately 100 μm in diameter were observed.

4. CONCLUSION AND OUTLOOK

We employed an x-ray generator with a 100- μm -focus tungsten tube and performed real-time magnification radiography (fluoroscopy) using the II camera. To perform angiography, we employed narrow-photon-energy bremsstrahlung x-rays with a peak photon energy of approximately 35 keV, which can be absorbed easily by iodine-based contrast media. The bremsstrahlung x-ray intensity substantially increased with increases in the tube voltage, and the tube voltage was determined as 60 kV in order to increase the image contrast by decreasing high-photon-energy bremsstrahlung x-rays with energies beyond 60 keV. In enhanced angiography, low-photon-energy bremsstrahlung rays should be absorbed by an aluminum filter. Although we obtained mostly absorption-contrast images, the phase-contrast effect may be added in cases where low-density media are employed.

We obtained spatial resolutions of approximately 110 μm using twofold magnification imaging using the II even when a 100- μm -focus tube was employed. In order to observe fine blood vessels of less than 100 μm , the spatial resolution of the radiography system should be improved to approximately 50 μm using the II driven in a high-resolution mode, and the iodine density should be increased. At a tube voltage of 60 kV and a current of 0.50 mA, the photon number was approximately 4×10^7 photons/($\text{cm}^2 \cdot \text{s}$) at 1.0 m from the source, and photon count rate can be increased easily using a rotating anode microfocus tube developed by Hitachi Medical Corporation. Because the focus diameter of the tube has been decreased to 10 μm , a high-resolution real-time magnification radiography system will become possible.

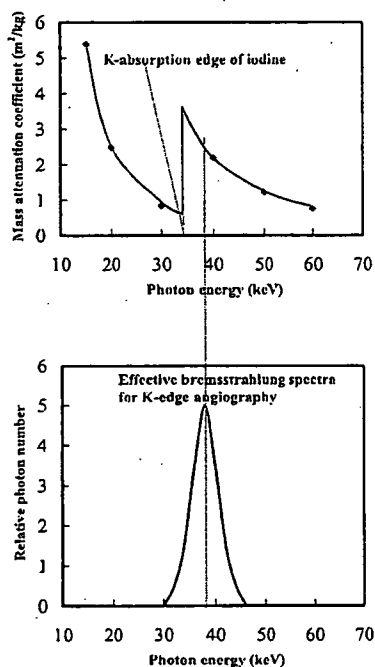


Fig. 8. Mass attenuation coefficients of iodine and effective bremsstrahlung x-rays for enhanced K-edge angiography.

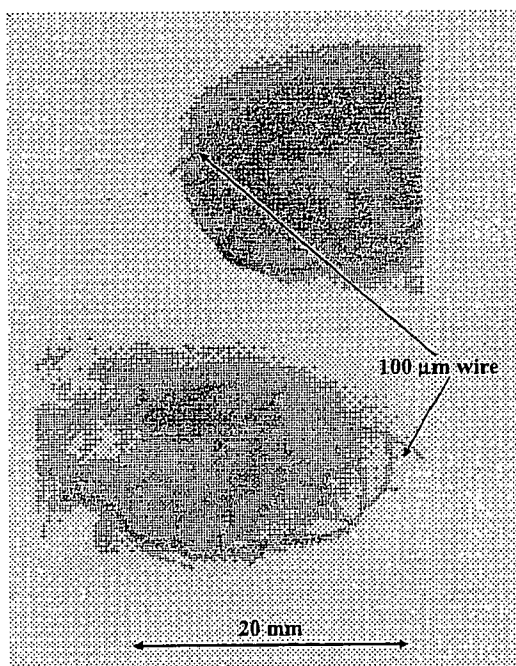


Fig. 9. Angiogram of an extracted rabbit heart using iodine microspheres.

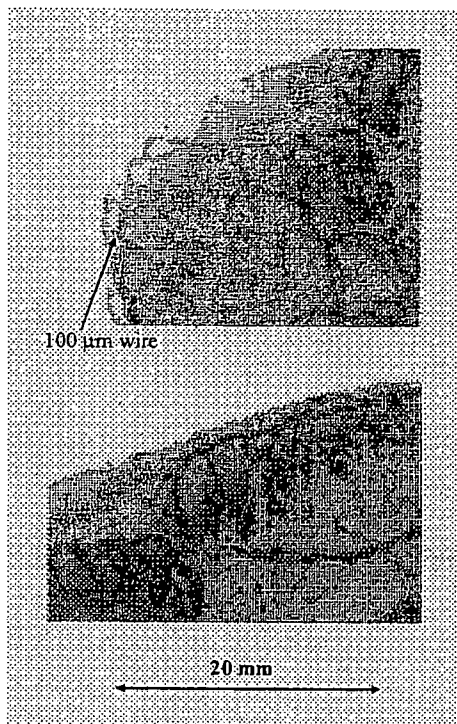


Fig. 10. Angiograms of an extracted dog heart.

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Demonstration of enhanced K-edge angiography utilizing a samarium x-ray generator

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ABSTRACT

The samarium-target x-ray tube is useful in order to perform cone-beam K-edge angiography because K-series characteristic x-rays from the samarium target are absorbed effectively by iodine-based contrast media. This generator consists of the following components: a constant high-voltage power supply, a filament power supply, a turbomolecular pump, and an x-ray tube. The x-ray tube is a demountable diode which is connected to the turbomolecular pump and consists of the following major devices: a samarium target, a tungsten hairpin cathode (filament), a focusing (Wehnelt) electrode, a polyethylene terephthalate x-ray window 0.25 mm in thickness, and a stainless-steel tube body. In the x-ray tube, the positive high voltage is applied to the anode (target) electrode, and the cathode is connected to the tube body (ground potential). In this experiment, the tube voltage applied was from 50 to 70 kV, and the tube current was regulated to within 0.10 mA by the filament temperature. The exposure time is controlled in order to obtain optimum x-ray intensity. The electron beams from the cathode are converged to the target by the focusing electrode, and bremsstrahlung x-rays were absorbed using a 50- μ m-thick tungsten filter. The x-ray intensity was 1.04 μ Gy/s at 1.0 m from the x-ray source with a tube voltage of 60 kV and a tube current of 0.10 mA, and angiography was performed using a computed radiography system and iodine-based microspheres 15 μ m in diameter. In angiography of non-living animals, we observed fine blood vessels of approximately 100 μ m with high contrasts.

Keywords: K-series characteristic x-rays, samarium target, demountable x-ray tube, enhanced K-edge angiography

1. INTRODUCTION

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Recent advances in x-ray technology aim at forming monochromatic parallel x-ray beams using synchrotrons in conjunction with silicon crystals. These beams have been applied in preliminary experiments for medical radiography including enhanced K-edge angiography^{1,2} using iodine media. In angiography, monochromatic x-rays with photon energies ranging from 33.3 to 35 keV have been employed because the rays are absorbed effectively by iodine-based contrast media with an iodine K-edge of 33.2 keV.

From weakly ionized linear plasmas³⁻⁶ of nickel and copper, extremely clean characteristic x-rays have been produced. In particular, we confirmed the irradiation of the second and fourth harmonic x-rays of the fundamental K-series characteristic x-rays from a copper target. The x-ray intensities of the harmonics increased with increases in the charging voltage, and the harmonic bremsstrahlung rays survived due to the x-ray resonance in the plasma.

Steady-state monochromatic x-ray generators⁷ have been developed to produce clean K-series characteristic x-rays utilizing the angle dependence of bremsstrahlung x-rays, since bremsstrahlung rays are not emitted in the opposite direction to that of electron trajectory. Subsequently, a cerium x-ray generator⁸⁻¹⁰ has been developed, and has been employed to perform enhanced K-edge angiography achieved with cerium K α rays and iodine-based contrast media, since K α rays (34.6 keV) are absorbed effectively by iodine. In this case, because the sampling pitch of a computed radiography system¹¹ (Konica Minolta Regius 150) is 87.5 μm , the spatial resolution of approximately 100 μm has been obtained.

To increase the K-series characteristic x-ray intensity, the tube current should be maximized at a constant tube voltage. Therefore, the melting temperature of the target element should be increased because the temperature of the cerium is 1072 K. In view of this situation, a samarium target can be employed, since the K α rays (39.9 keV) from a samarium target are also absorbed effectively by iodine, and the melting temperature is 1350 K.

In the present research, we developed a new samarium x-ray generator and performed a preliminary study on enhanced K-edge angiography achieved with samarium K-series characteristic x-rays.

2. GENERATOR

Figure 1 shows a block diagram of a compact characteristic (quasi-monochromatic) x-ray generator. This generator consists of the following components: a constant high-voltage power supply (SL150, Spellman), a DC filament power supply, a turbomolecular pump, and an x-ray tube. The x-ray tube is a demountable diode which is connected to the turbomolecular pump with a pressure of approximately 0.5 mPa and consists of the following major devices: a samarium rod target of 6.5 mm in diameter, a tungsten hairpin cathode (filament), a focusing (Wehnelt) electrode, a polyethylene terephthalate x-ray window 0.25 mm in thickness, and a stainless-steel tube body (Fig. 2). In the x-ray tube, the positive high voltage is applied to the anode (target) electrode, and the cathode is connected to the tube body (ground potential). In this experiment, the tube voltage applied was from 50 to 70 kV, and the tube current was regulated to within 0.10 mA by the filament temperature. The exposure time is controlled in order to obtain optimum x-ray intensity. The electron beams from the cathode are converged to the target by the focusing electrode, and x-rays are produced through the focusing electrode.

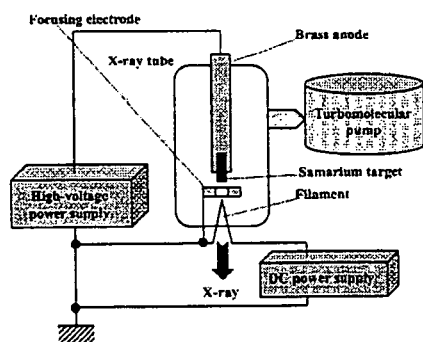


Fig. 1. Block diagram of the x-ray generator with a samarium-target radiation tube, which is used specially for K-edge angiography using iodine-based contrast media.

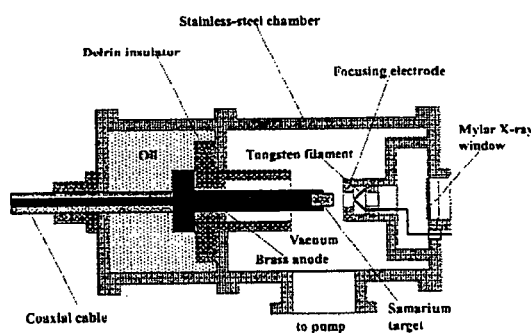


Fig. 2. Structure of the x-ray tube with a samarium target

3. CHARACTERISTICS

3.1 X-ray intensity

The x-ray intensity was measured by a Victoreen 660 ionization chamber at 1.0 m from the x-ray source using a 50- μm -thick tungsten filter (Fig. 3). At a constant tube current, the x-ray intensity increased when the tube voltage was increased. At a tube voltage of 60 kV and a current of 0.10 mA, the intensity with the filter was 1.04 $\mu\text{Gy/s}$.

3.2 Focal spot

In order to measure images of the x-ray source, we employed a pinhole camera with a hole diameter of 100 μm in conjunction with a Computed Radiography (CR) system with a sampling pitch of 87.5 μm (Fig. 4). When the tube voltage was increased using the filter, the spot diameter increased and had a maximum value of approximately 2.2 mm with a tube voltage of 70 kV.

3.3 X-ray spectra

In order to measure x-ray spectra, we employed a cadmium telluride detector (XR-100T, Amptek) (Fig. 5). Using the filter, low-photon-energy bremsstrahlung x-rays were absorbed, and sharp K lines were left. When the tube voltage was increased, the x-ray intensities of samarium K-series characteristic lines increased, and the maximum photon energy increased.

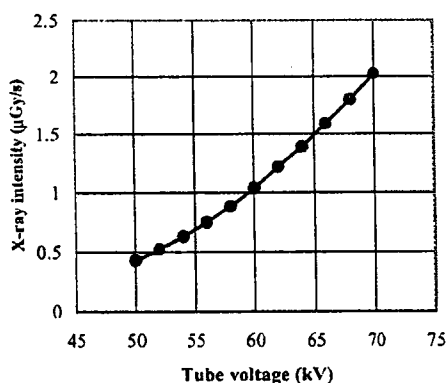


Fig. 3. X-ray intensity measured at 1.0 m from X-ray source according to changes in tube voltage using a tungsten filter.

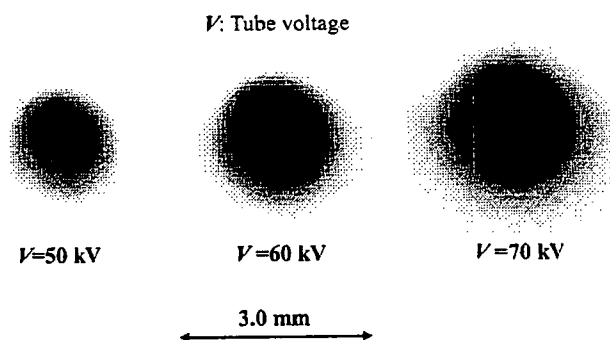


Fig. 4. Images of the x-ray source with changing the tube voltage.

4. K-EDGE ANGIOGRAPHY

Because the average photon energy of samarium $K\alpha$ is 39.9 keV, iodine contrast media with a K-absorption edge of 33.2 keV absorb the $K\alpha$ lines easily (Fig. 6). Therefore, blood vessels were observed with high contrasts. The angiography was performed using the CR system, iodine microspheres of 15 μm in diameter, and the filter. The distance between the x-ray source and the imaging plate was 1.0 m, and the tube voltage was 60 kV. First, rough measurements of spatial resolution were made using wires coiled around rods made of polymethyl methacrylate (PMMA) (Fig. 7). Although the image contrast decreased somewhat with decreases in the wire diameter, a 50- μm -diameter wire could be observed.

Figures 8 and 9 show angiograms of a rabbit heart and thigh, respectively. The coronary arteries in the heart and fine blood vessels in the thigh were visible. Figure 10 shows angiograms of a dog heart, and blood vessels of approximately 100 μm in diameter were observed.

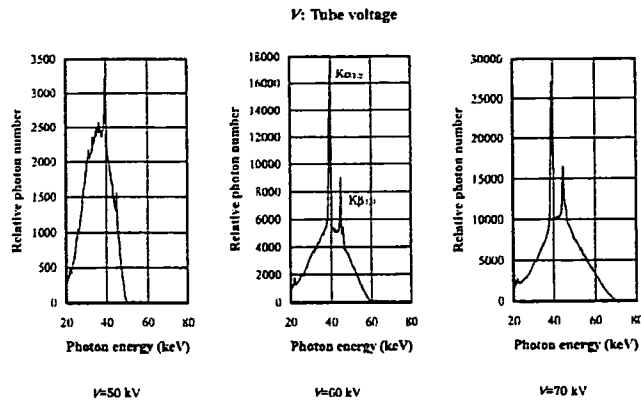


Fig. 5. X-ray spectra measured using a cadmium telluride detector with changes in tube voltage using the filter.

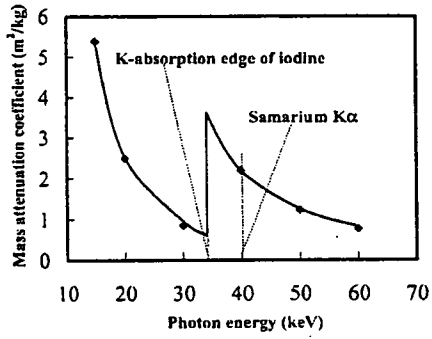


Fig. 6. Mass attenuation coefficients of iodine and average photon energy of samarium $K\alpha$ lines.

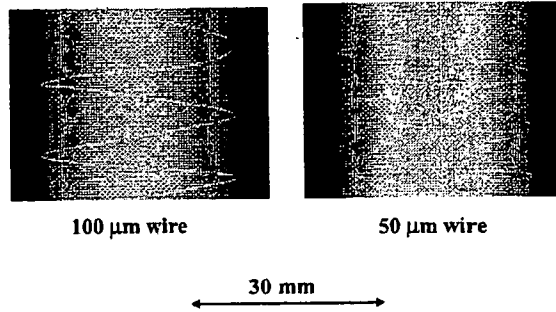


Fig. 7. Radiograms of tungsten wires coiled around PMMA rods.

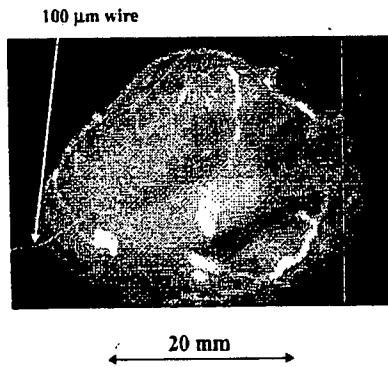


Fig. 8. Angiograms of an extracted rabbit heart using iodine microspheres.

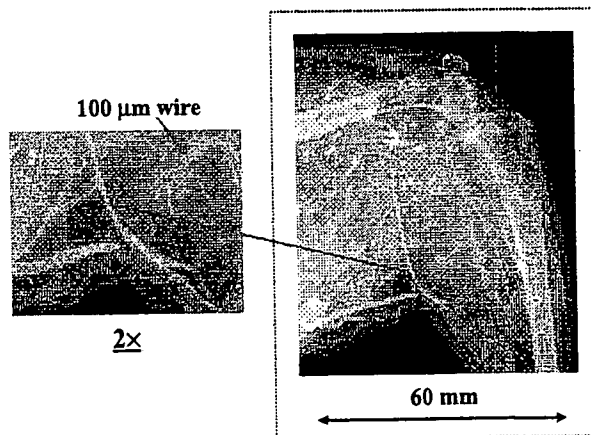


Fig. 9. Angiogram of a rabbit thigh using iodine microspheres.

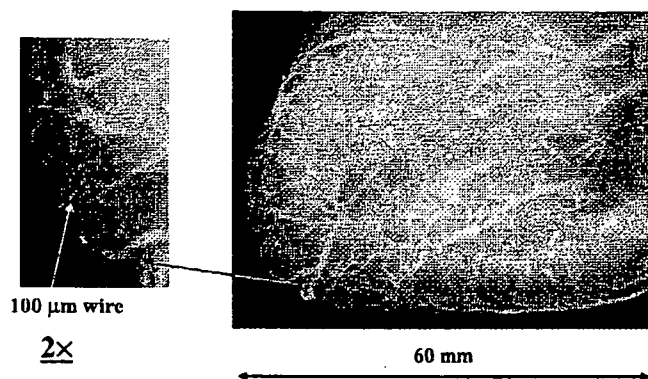


Fig. 10. Angiogram of an extracted dog heart using iodine microspheres.

5. DISCUSSION AND CONCLUSIONS

We employed an x-ray generator with a samarium-target tube and succeeded in producing samarium characteristic x-rays, which can be absorbed easily by iodine-based contrast media. Both the characteristic and bremsstrahlung x-ray intensities increased with increases in the tube voltage without filtering. Using the filter, K-rays were left by absorbing bremsstrahlung rays, and K-ray intensity increased with increases in the tube voltage.

Using this x-ray tube, we could produce K-series characteristic x-rays of nickel, copper, and molybdenum, and performed soft radiography. However it is difficult to produce clean samarium K-rays because bremsstrahlung x-ray intensity is in proportion to the atomic number. Therefore, optimum filters for absorbing bremsstrahlung rays should be employed to improve the image contrast of blood vessels.

Using the filter, the generator produced maximum number of characteristic photons was approximately 4×10^6 photons/(cm²·s) at 1.0 m from the source, and the photon count rate can be increased easily by improving the target. For example, the rotation anode tube can be developed, and sufficient x-ray dose rates could be produced by increasing the anode diameter.

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Super-characteristic x-ray generator utilizing a pipe and rod target

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ABSTRACT

This generator consists of the following components: a constant high-voltage power supply, a filament power supply, a turbomolecular pump, and an x-ray tube. The x-ray tube is a demountable diode which is connected to the turbomolecular pump and consists of the following major devices: a tungsten hairpin cathode (filament), a focusing (Wehnelt) electrode, a polyethylene terephthalate x-ray window 0.25 mm in thickness, a stainless-steel tube body, a pipe target, and a rod target. The pipe and rod targets are useful for forming linear and cone beams, respectively. In the x-ray tube, the positive high voltage is applied to the anode (target) electrode, and the cathode is connected to the tube body (ground potential). In this experiment, the tube voltage applied was from 12 to 20 kV, and the tube current was regulated to within 0.10 mA by the filament temperature. The exposure time is controlled in order to obtain optimum x-ray intensity. The electron beams from the cathode are converged to the target by the focusing electrode, and clean K-series characteristic x-rays are produced through the focusing electrode without using a filter. The x-ray intensities of the pipe and rod targets were 1.29 and 4.28 $\mu\text{Gy/s}$ at 1.0 m from the x-ray source with a tube voltage of 15 kV and a tube current of 0.10 mA, and quasi-monochromatic radiography was performed using a computed radiography system.

Keywords: demountable x-ray tube, electron-impact source, line beam, cone beam, quasi-monochromatic x-rays, K-series characteristic x-rays, Sommerfeld's theory

1. INTRODUCTION

Gas-discharge capillaries play significant roles in irradiation of soft x-ray lasers,¹⁻³ and the laser photon energy has been increasing. Subsequently, large-scale x-ray free electron laser sources⁴ are constructing as a new-generation radiation source for producing monochromatic coherent x-rays to perform various research projects including biomedical applications. However, it is quite difficult to increase the maximum photon energy to 10 keV or beyond.

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To produce short x-ray pulses, several various flash x-ray generators utilizing high-voltage condensers have been developed, and high-speed radiography has been demonstrated. In particular, the importance of forming weakly ionized plasma source⁵⁻⁸ is well reported to produce clean K-series characteristic x-rays, and the second and fourth harmonic x-rays of the fundamental K-series characteristic x-rays from copper and nickel targets have been confirmed. The x-ray intensities of the harmonics increase with increases in the charging voltage, and the harmonic bremsstrahlung rays survive due to the x-ray resonance in the plasma. However, it is not easy to produce high-photon-energy K-rays using linear plasmas, since the plasmas readily transmit high-photon-energy bremsstrahlung x-rays. In view of this situation, we have developed new flash x-ray generators⁹⁻¹¹ to produce high-photon-energy K-rays of molybdenum, cerium, tantalum, and tungsten.

At present, monochromatic parallel x-ray beams from synchrotrons utilizing silicon crystals are used in various fields including medical imaging. In particular, x-rays with photon energies ranging from 33.3 to 35 keV have been employed to perform enhanced K-edge angiography^{12,13} because the rays are absorbed effectively by iodine-based contrast media with an iodine K-edge of 33.2 keV. This imaging plays significant roles in the diagnosis of coronary arteries, fine blood vessels in regenerative medicine, and capillaries in tumors. In contrast, small-scale steady-state monochromatic parallel and cone beams¹⁴⁻¹⁶ can be employed to perform medical imaging in hospitals.

In this research, we developed an x-ray generator used to perform a preliminary experiment for generating clean K-series characteristic x-rays using a pipe and rod target by angle dependence of the bremsstrahlung x-rays.

2. GENERATOR

Figure 1 shows a block diagram of a compact characteristic (quasi-monochromatic) x-ray generator. This generator consists of the following components: a constant high-voltage power supply (SL150, Spellman), a DC filament power supply, a turbomolecular pump, and an x-ray tube. The structures of the x-ray tube are illustrated in Figs. 2 and 3. The x-ray tube is a demountable diode which is connected to the turbomolecular pump with a pressure of approximately 0.5 mPa and consists of the following major devices: a tungsten hairpin cathode (filament), a focusing (Wehnelt) electrode, a polyethylene terephthalate x-ray window 0.25 mm in thickness, a stainless-steel tube body, a rod copper target of 3.0 mm in diameter, and a pipe copper target with an outside and a bore diameters of 5.0 and 4.0, respectively. In the x-ray tube, the positive high voltage is applied to the anode (target) electrode, and the cathode is connected to the tube body (ground potential). In this experiment, the tube voltage applied was from 12 to 20 kV, and the tube current was regulated to within 0.10 mA by the filament temperature. The exposure time is controlled in order to obtain optimum x-ray intensity. The electron beams from the cathode are converged to the target by the focusing electrode, and x-rays are produced through the focusing electrode. Because bremsstrahlung rays are not emitted in the opposite direction to that of electron trajectory in Sommerfeld's theory²⁰ (Figs. 4 and 5), clean molybdenum K-series x-rays can be produced without using a filter.

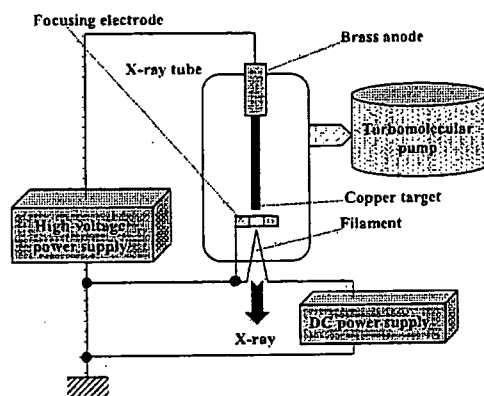


Fig. 1. Block diagram including the main transmission line of the compact x-ray generator with a quasi-monochromatic diode.

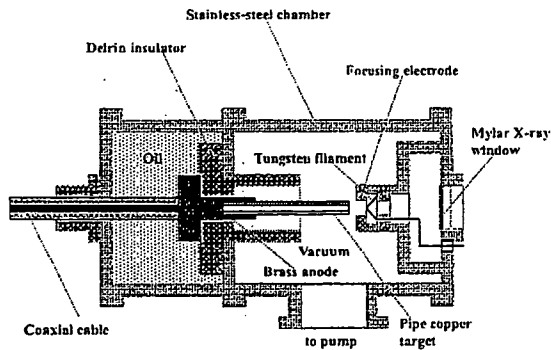


Fig. 2. Schematic drawing of the characteristic x-ray tube with a pipe copper target

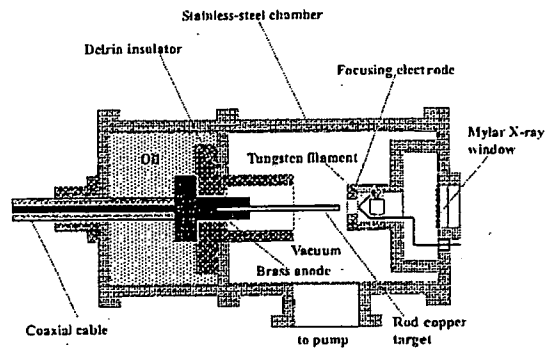


Fig. 3. Structure of the characteristic x-ray tube with a rod copper target

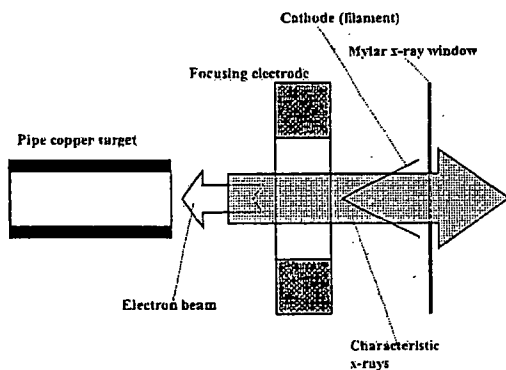


Fig. 4. K-photon irradiation from the pipe-target x-ray tube.

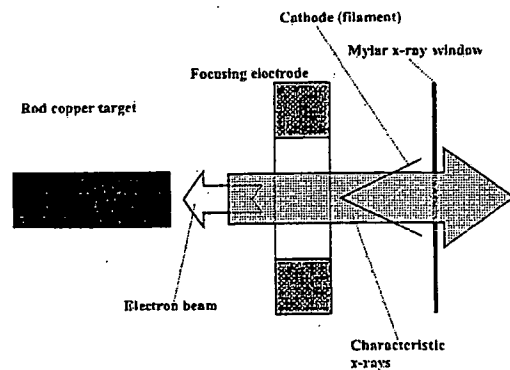


Fig. 5. K-photon irradiation from the rod-target x-ray tube.

3. CHARACTERISTICS

3.1 X-ray intensity

X-ray intensities from the pipe and rod targets were measured by a Victoreen 660 ionization chamber at 1.0 m from the x-ray source (Figs. 6 and 7). At a constant tube current of 0.10 mA, the x-ray intensity increased when the tube voltage was increased. In this measurement, the intensities of the pipe and rod targets were 1.29 and 4.28 $\mu\text{Gy/s}$, respectively, at 1.0 m from the source with a tube voltage of 15 kV.

3.2 X-ray source

In order to measure images of the x-ray sources, we employed a pinhole camera with a hole diameter of 100 μm in conjunction with a computed radiography (CR) system (Figs. 8 and 9). When the tube voltage was increased using the pipe target, the spot intensities increased, and the maximum diameter was equal to the bore diameter. On the other hand, both the intensity and diameter increased with increases in the tube voltage, and the maximum diameter was approximately 2.2 mm.

3.3 X-ray spectra

X-ray spectra were measured using a silicon detector (XR-100CR, Amptek). We observed sharp K lines, and the characteristic x-ray intensities substantially increased with increases in the tube voltage (Figs. 10 and 12). Clean K lines were left by a 10- μm -thick copper filter, and the $K\alpha$ lines were selected out by absorbing $K\beta$ lines using a 10- μm -thick nickel filter (Figs. 11 and 13).

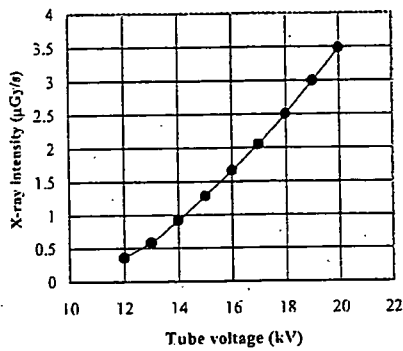


Fig. 6. X-ray intensity at 1.0 m from the pipe target according to changes in the tube voltage with a tube current of 0.10 mA.

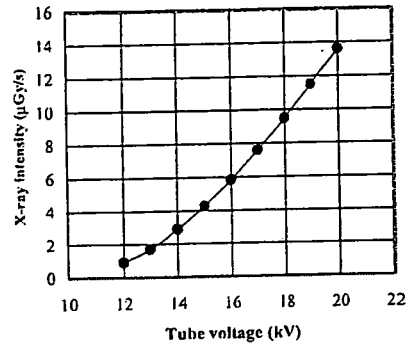


Fig. 7. X-ray intensity at 1.0 m from the rod target with changing the tube voltage with a tube current of 0.10 mA.

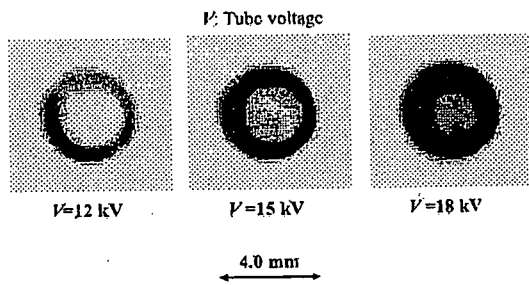


Fig. 8. Images of the characteristic x-ray source from the pipe target with changes in the tube voltage.

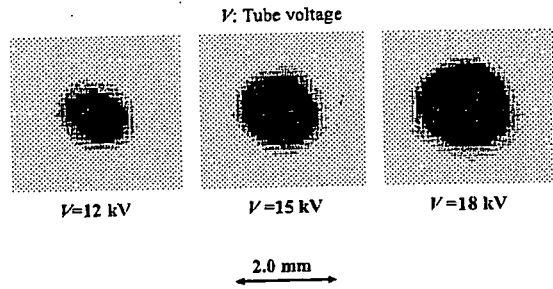


Fig. 9. Images of the characteristic x-ray source from the rod target.

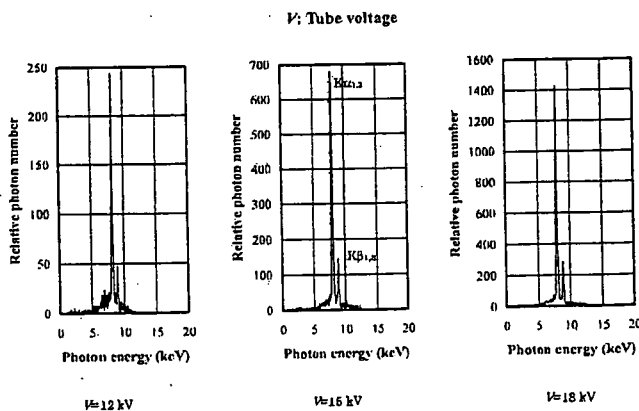


Fig. 10. X-ray spectra from the pipe target with changes in the tube voltage.

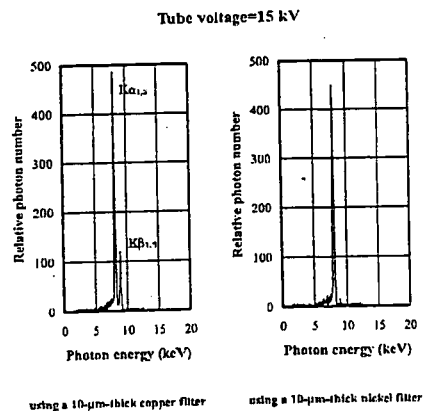


Fig. 11. X-ray spectra from the pipe target according to insertion of filters.

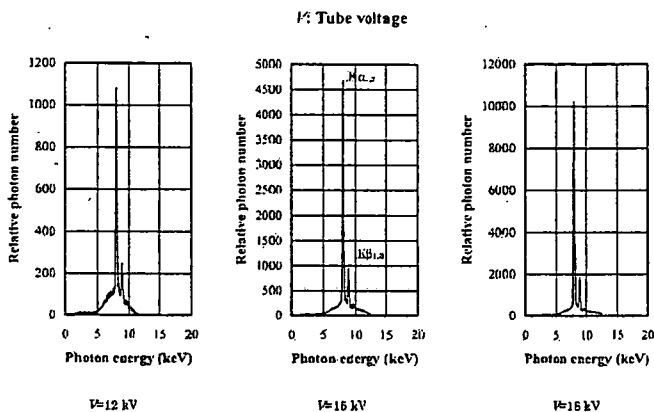


Fig. 12. X-ray spectra from the rod target with changing the tube voltage

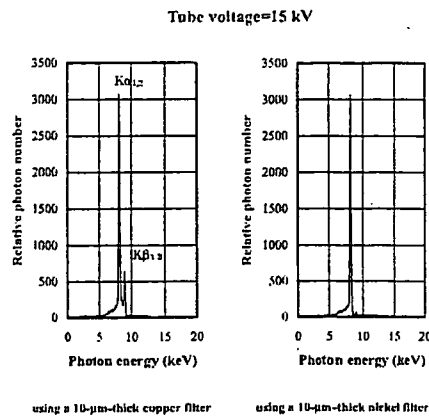


Fig. 13. X-ray spectra from the rod target according to insertion of filters.

4. CONCLUSIONS AND OUTLOOK

We developed a super-characteristic x-ray generator with the pipe and rod targets and succeeded in producing copper K lines. The characteristic x-ray intensity increased with increases in the tube voltage, and monochromatic K α lines were left by the nickel filter.

In the spectrum measurements, we usually employ a silicon detector and a lithium fluoride curved crystal. The detector is useful for measuring the total spectra, including scattering beams. On the other hand, the spectra from only the x-ray source can be measured using the crystal by selecting Bragg's angle. Using the crystal in conjunction with a computed radiography system, we observed clean copper K lines.

In this preliminary experiment, although the maximum tube voltage and current were 20 kV and 0.10 mA, the voltage and current could be increased to 100 kV and 1.0 mA, respectively. Using the rod target, the generator produced maximum number of characteristic photons from the rod target was approximately 1×10^6 photons/(cm²·s) at 1.0 m from the source, and the photon count rate can be increased easily by increasing the current.

Currently, the copper K-series characteristic x-rays are useful for extremely soft radiography, and the photon energies of characteristic x-rays can be selected by the target element. In particular, the pipe target is useful for forming monochromatic line beams by decreasing the bore diameter.

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Efficient Preparation of Cationized Gelatin for Gene Transduction

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We previously reported gene therapy using cationized gelatin microspheres of ϕ 20-32 μ m, prepared from pig skin, as a transducing agent, but although the gelatin offered various advantages, its yield was extremely low (only 0.1%). In this study, we markedly improved the yield of ϕ 20-32 μ m cationized gelatin microspheres and prepared a newly less than ϕ 20 μ m cationized gelatin. Conventionally, cationized gelatin is prepared by cationization, particulation by agitation, and cross-linking. The yield is determined by the particulation step, for which we had used a three-necked distillation flask of 500 mL and an agitation speed of 420 rpm. The yield was significantly increased from $0.13 \pm 0.02\%$ to $8.80 \pm 1.90\%$ by using a smaller flask of 300 mL and an agitation speed of 25000 rpm ($p < 0.01$). We could also prepare cationized gelatin of less than ϕ 20 μ m, which had not been possible previously. We confirmed that efficient gene introduction into peritoneal macrophages could be achieved with the new cationized gelatin.

Key words: gelatine microsphere, macrophage, yield

INTRODUCTION

Efficient gene transduction methods are necessary for gene therapy [1], and currently available methods can be divided into viral vector techniques and non-viral approaches, such as lipofection or electroporation. Viral vectors such as adenovirus or retrovirus offer high transduction efficiency, but there are questions regarding safety [2]. On the other hand, the efficiency of transduction with non-viral vectors is generally poor [3]. In recent years, nucleofection has been developed for highly efficient gene transduction, but it can be applied to only certain cells, and it causes damage in some cases [4].

We showed that intramuscular injection of FGF-4 gene-gelatin complex induced significantly greater angiogenesis than injection of the bare FGF-4 gene [5]. Furthermore, we showed that adrenomedullin (AM) gene-gelatin complex effectively transduced the AM gene into endothelial progenitor cells (EPCs), and the transduced EPCs had a therapeutic effect in pulmonary hypertension [6]. In those studies, we used cationized gelatin microspheres of ϕ 20-32 μ m, derived from pig skin. However, the yield of gelatin was only about 0.1%. Cascone *et al.* reported that the preparation of nanoparticulate gelatin required an agitation speed of cationized gelatin and olive oil of more than 10,000 rpm [7], so in this study, we examined whether the use of a higher agitation speed during preparation of the gelatin particles would increase the yield and reduce the particle size in our procedure. We also confirmed the efficacy of the gelatin particles thus obtained for gene transduction.

MATERIALS

Gelatin of pig skin origin (PI 9) was purchased from Nitta Gelatin Corp, Japan. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), β -alanine, acetone, glycine, hydrochloric acid, ethylenediamine, olive oil, glutaraldehyde solution (GA), potassium dihydrogenphosphate, disodium hydrogenphosphate and sodium hydrogen carbonate were purchased from Wako Pure Chemical Industry, Japan. Liquid nitrogen was purchased from Tomoe Corporation, Japan.

Rat peritoneal macrophages were collected by intraperitoneal injection of thioglycolate culture medium as previously described [8]. DNA encoding GFP with the cytomegalovirus enhancer-chicken β -actin hybrid promoter was constructed [5].

METHODS

Conventional preparation of gelatin microspheres involves three steps: (1) cationized gelatin production, (2) microsphere production and (3) cross-linking of cationized gelatin microspheres. In order to increase the yield of cationized gelatin microspheres, we aimed to improve the second step, i.e., microsphere production.

Cationized gelatin production

PI 9 (10 g) was completely dissolved in 0.1 M phosphate buffer (PBS, 450 mL) containing potassium dihydrogenphosphate and disodium hydrogenphosphate. Ethylenediamine (31.1 mL) and hydrochloric acid were added, and the pH was adjusted to 5.0. EDC (5.35 g) was added to this solution, which was made up to 500 mL with PBS and left for 18 hours. The solution was