A Device for In Vitro Irradiation with $\alpha$-Particles

Using an $\alpha$- Emitting Radioactive Source

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A device to irradiate a monolayer of cultured cells with $\alpha$-particles using an Am-241 $\alpha$-source (33.4 MBq) was designed to investigate RBEs of $\alpha$-particles in cell killing, induction of chromosome aberration, mutagenic changes and transformation. This device can be used conveniently in a common laboratory by a small number of researchers without any limitation of machine time. The device performs as follows: (1) The energy of $\alpha$-particles at the entrance of the cell layer is 3.20 MeV with a standard deviation of 0.25 MeV, (2) the incident angle to the cell layer is 82.8 degrees with a standard deviation of 3.2 degrees, (3) the fluence rate is $4.7 \times 10^{9}$ cm$^{-2}$·min$^{-1}$, (4) the average LET$\alpha$ for a cell layer 5 $\mu$m thick is 138 keV/$\mu$m, (5) the average dose rate for a cell layer 5 $\mu$m thick is 0.10 Gy/min., (6) a temperature and CO$_2$ concentration conducive to cell cultivation are maintained during irradiation.

INTRODUCTION

Cell studies by $\alpha$-particle irradiation have played an important role in understanding the early changes of biological systems exposed to high LET radiation. These studies are also expected to bring a scientific basis to the assessment of the risk associated with the intake of $\alpha$-emitting radionuclides such as radon progeny or nuclear fuels.

Dufrain et al.$^{1}$ and Purrot et al.$^{2}$ investigated cytogenetic changes in lymphocytes by adding solutions of $\alpha$-emitting radionuclides to blood samples. Blood cell dosimetry in such an exposure medium is a complicated task. External irradiation of $\alpha$-particles to cultured cells, on the other hand, promises a more simple and reliable cell dosimetry. Particle accelerators$^{3-5}$ and solid sources of $\alpha$-emitting radionuclides$^{6-19}$ were used for external sources. A device using a solid $\alpha$-source, compared with particle accelerators, is limited in the number of types of charged particles it produces, and in the variability of their energies and particle fluence rates. However, the device can be used conveniently in a common laboratory by a small number of researchers without any limitation of machine time. Recently, several devices were developed which can bring
IN VITRO IRRADIATION WITH $\alpha$-PARTICLES

a precise determination and well defined delivery of absorbed dose to the cell culture system$^{20,22}$. In the present work we designed and constructed an irradiation device based on a somewhat different design than these devices.

The following important criteria were considered in designing the device.

1. The $\alpha$-particles have an energy sufficiently high to penetrate through a monolayer of cultured cells.

2. Because the energy of $\alpha$-particles influences the response of cells to the radiation, the broadening of $\alpha$-particle energy is restricted to an acceptable level.

3. To equalize the path length of each $\alpha$-particle traversing the cell layer, the incident angle of the $\alpha$-particles is made uniform.

4. The fluence rate is uniform over the irradiated area.

5. The dose rate is reasonably high in order to accomplish the irradiation in a short time.

6. The cell cultivation conditions, especially the temperature and the CO$_2$ concentration of the atmosphere, are maintained during irradiation.

MATERIALS AND METHODS

A schematic diagram of the device is shown in Fig. 1.

The device is wholly housed within a CO$_2$ incubator to maintain the atmospheric conditions conducive to cell culture.

Cells are cultivated on a thin sheet of polyester foil which forms the base of specially made culture dish (as shown in Fig. 2). The thickness of the foil is equivalent to a water layer of 6 $\mu$m. A shutter system, which requires a substantial air space between the source and the target, was not adopted in the present device, because the air space degrades the energy of $\alpha$-particles and broadens their distribution. Instead of using a shutter system the initiation/termination of the irradiation is carried out as follows. Before irradiation, the dish is placed beyond the range of the $\alpha$-particles from the source. The irradiation is initiated by lifting down the dish and holding it close to the source. When the irradiation is terminated, the dish is lifted up to a position out of range of the $\alpha$-particles. These quick up/down movements are precisely made by a pulse motor under control of a microprocessor.

A thin ceramic plate, 0.8 mm in thickness, was used as a collimator to minimize the source to target distance. Ten thousand holes 0.22 mm in diameter spaced 0.3 mm apart were drilled in the ceramic plate by laser beam. The source to target distance was minimized so as to average 3 mm.

The $\alpha$-source is a commercially available Am-241 sealed source (Radiochemical Center Amersham, UK), whose active area is 30mm x 30mm. The surface of the source is covered with a thin layer of gold-palladium alloy. The total activity of the source is nominally 33.4 MBq.

The source and the collimator are held on an optical microscope scanning stage unit (Microscanner, Sapporo Breweries Ltd., Tokyo, Japan), which is driven microscopically in the X- and Y-directions by a pulse motor under control of a microprocessor. The fluence rate of $\alpha$-particles at the cell position can be made uniform by moving the scanning stage translationally during
Fig. 1. Diagram of the device designed in the present work.

Fig. 2. Diagram of a culture dish placed at the position of irradiation.

irradiation.

The fluence rate of the $\alpha$-particles and their uniformity over the irradiated area were determined by measuring the spatial distribution of $\alpha$-tracks registered on a solid state nuclear track detector placed at the same position as the target.
RESULTS AND DISCUSSION

1. Performance of the collimator

The incident angle ($\theta$) of $\alpha$-particles passing through the collimator and their relative path lengths in the cell monolayer ($1/\sin\theta$) were estimated by Monte-Carlo simulation (Fig. 3(a) and Fig. 3(b), respectively). It was found that the collimator restricts the $\alpha$-particles to an angle larger than $74^\circ$; the average of the angles is $82.8^\circ$ and the standard deviation $3.2^\circ$. The relative path length is shown to be almost unity: the average is 1.009 and the standard deviation 0.007.

2. Energy distribution of $\alpha$-particles

The energy distribution of $\alpha$-particles emitted from the Am-241 source was measured in a vacuum chamber with a silicon surface barrier detector. The energy spectrum is shown in Fig. 4 by open circles. The average energy was found to be 4.14 MeV with a standard deviation of 0.20 MeV. When a sheet of the base foil was placed between the source and the detector, the energy was degraded to 3.57 MeV. The 3 mm air space between the source and the cell target also absorbs $\alpha$-particle energy. Air was introduced into the vacuum chamber up to a pressure equivalent to 1 atm for the 3 mm air space. The energy spectrum measured at this pressure is shown by closed circles in Fig. 4. It was found that the average energy of the $\alpha$-particles at the entrance to the cultured cells was 3.20 MeV with a standard deviation of 0.25 MeV.

![Fig. 3. Performance of the collimator: distribution of (a) the incident angles and (b) the relative path lengths of $\alpha$-particles impinging on the cell layer.](https://academic.oup.com/jrr/article-abstract/32/4/404/938178)
3. Fluence rate and its uniformity

The track etch method was applied to measure the fluence rate and its uniformity. A sheet of solid state nuclear track detector CR-39 (BARYOTRAK from Nagase-Landauer, Tokyo, Japan) was used. The tracks of $\alpha$-particles impinging on the CR-39 plate are enlarged by chemical etching so as to become visible under an optical microscope as shown in Fig. 5. These enlarged tracks are usually called etch pits.

All $\alpha$-particles impinging on the plate whose incident angles are larger than 74°, can be counted as etch pits, because the incident angle critical for etch pit formation is smaller than 25°. From the number of etch pits per unit of area of the CR-39 plate irradiated at the same position as of the cell layer, the fluence rate was found to be $4.7 \times 10^5 \text{ cm}^{-2}\cdot\text{min}^{-1}$.

The survival curves that would be expected for irradiation of this fluence rate were estimated for different reaction cross sections as shown in Fig. 6.

The uniformity of the fluence was investigated by checking whether the $\alpha$-particle incidence is Poisson process or not. Four hundred squares of small area ($25 \mu\text{m} \times 25 \mu\text{m}$) were randomly picked over the surface of the irradiated plate. The frequency distribution for the number of etch pits counted in each square was obtained as shown in Fig. 7; the average number of etch pits was 8.8/square. The Poisson distribution for the same average value was calculated and superimposed on the measured frequency distribution in Fig. 7, showing that the measured distribution closely fitted the Poisson distribution.
Fig. 5. Etched CR-39 plate that was irradiated with α-particles at the cell layer position.

Fig. 6. Expected survival curves for different action cross sections.
Fig. 7. Uniformity of fluence rate at the cell layer position: Frequency distribution of the number of tracks counted in each 25 μm × 25 μm square on the irradiated CR-39 plate. Randomness of the spatial distribution of incident positions of α-particles was verified as a result of the closeness of the fit between the measured frequency distribution and calculated Poisson distribution.

The uniformity of activity on the source plane was also investigated by the track etch method. The entire irradiated area was dissected into 25 squares of 5mm × 5mm and the etch pit density measured for each square. The spatial distribution of the etch pit densities is illustrated in Fig. 8. The distribution was within the range of statistical deviation of etch pit counts.

4. Dose rate and LET, and their dependences on depth

The absorbed dose varies according to the depth in the target cells, because the LET (linear energy transfer) of each α-particle increases with depth up to a position near the range limit and also because the ranges of α-particles are not all the same. The distributions of dose rate and LET were calculated based on the measured values of the flux density and energy distribution of α-particles.

The absorbed dose at depth t, D(t), is given by

\[ D(t) = C \int_{E_{\text{min}}}^{E_{\text{max}}} f(E) \text{LET}_\alpha(t,E) dE, \]  

(1)
The uniformity of radioactivity on the source plane: spatial distribution of each pit densities on a CR-39 plate.

where

- $E$: $\alpha$-particle energy upon entrance of the cell layer,
- $E_{\text{min}}$: beam minimum energy,
- $E_{\text{max}}$: beam maximum energy,
- $C$: constant to convert to absorbed dose,
- $f(E)dE$: particle fluence between $E$ and $E + dE$,
- $\text{LET}_\infty(t,E)$: linear energy transfer at depth $t$ of $\alpha$-particles whose energy upon entrance of the cell layer is $E$.

The value of $\text{LET}_\infty(t)$ averaged for the $\alpha$-particles that can reach depth $t$ is given by

$$\text{LET}_\infty(t) = \frac{\int_{E_0}^{E_{\text{max}}} f(E) \, \text{LET}_\infty(t,E) \, dE}{\int_{E_0}^{E_{\text{max}}} f(E) \, dE},$$

where $E_0$ is the lowest energy (upon entrance of the cell layer) among the $\alpha$-particles that can reach depth $t$. When all particles in the beam reach depth $t$, $E_0$ is equal to $E_{\text{min}}$.

The values of $\text{LET}_\infty$ are available from the literature$^{23-25}$. By using these published data
the depth dependences of LET∞ were calculated. The results in the case of 3.2 MeV are shown in Fig. 9. The LET∞ values are nearly the same at a depth of less than 10 μm, which is greater than the typical thickness of cells attached to a plane. We used the stopping power values tabulated in the ICRU REPORT 36 to calculate LET∞(t) and D(t) for the beam available in the present device by approximating the values tabulated by polynomial functions of the α-particle energy. The result for dose rate is shown in Fig. 10 and for LET∞ in Fig. 11. The average dose rate for a cell layer 5 μm thick is 0.10 Gy/min and LET∞ is 138 keV/μm, and for a cell layer 10 μm thick, 0.11 Gy/min and 154 keV/μm are obtained, respectively.

The fraction of absorbed dose due to photons from Am-241 (γ-rays and LX-rays of Np) was estimated by calculation and found to be less than 0.03% of the α-particle dose.

5. Comparison of the performance of the present device with that of other devices

As mentioned in the introductory chapter, Roos et al. recently developed a useful device.
Fig. 10. Dependence of absorbed dose on the depth of soft tissue for the beam available in the present device, by approximating the values tabulated in ICRU REPORT 36 by a polynomial function of $\alpha$-particle energy.

Fig. 11. Dependence of LET$_{\alpha}$ on the depth of soft tissue for the beam available in the present device, by approximating the values tabulated in ICRU REPORT 36 by a polynomial function of $\alpha$-particle energy.
for cell irradiation. Inkret et al.\textsuperscript{22}) also independently developed an excellent device based on a different structure. The performance of these devices and ours is shown in Table 1 for comparison.

**Energy:**

The uniformity of energy is better in the device by Inkret et al. than in the other two devices. This is because Inkret used a very thin electrodeposited \(\alpha\)-source, which avoids the diversity of energy observed in a sealed source that is due to the energy straggling in the source seal material and to the nonuniformity of its thickness. Though an open source is thus preferable for uniformity of energy, the handling of a strong open source of \(\alpha\)-emitters is not convenient from the viewpoint of radiation protection. Because a uniformity sufficient for radiobiological study was confirmed in the present device, we preferred the convenience in handling to improved energy uniformity. In the device by Roos et al. the energy distribution was intentionally broadened to achieve a constant dose to the target cells.

**Incident angle:**

As regards beam collimation, the performance of the present device falls between that of the other two devices. The excess path length through the cell layer of an \(\alpha\)-particle most obliquely incident to the cell layer is 2.2% in the device of Roos et al., 4.0% in the present device and 7.9% in the device of Inkret et al.

**Uniformity of fluence:**

Roos et al. achieved a highly uniform fluence rate by rotating the source and moving the collimator circularly. In the device by Inkret et al., on the other hand, the source, the collimator

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<th>Table 1. Comparison of the performances of the present device with those by Roos et al.\textsuperscript{21}) and Inkret et al.\textsuperscript{22})</th>
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<td>Energy (S.D.)</td>
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<td><strong>Roos, H. et al.</strong></td>
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<td><strong>Inkret, W. et al.</strong></td>
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<td><strong>Present</strong></td>
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\(\ast\) Energy distribution was intentionally broadened to compensate for the depth dependence of the absorbed dose.

\(^{**}\) Calculated by the authors from the reported data for the dimensions of the collimator.
and the target are all static, which causes a standard deviation three times larger than that expected from the Poisson process. In the present device the simple translational motion of the source/collimator gives a highly uniform fluence to the cell layer.

Dose rate

The device by Inkret et al. gives a very high dose rate, which is achieved by a strong $\alpha$-source and a highly transparent collimator. Though high dose rate reduces the irradiation time, close control of irradiation time is required for precise irradiation. Therefore, a sophisticated shutter system must be incorporated as in Inkret’s device.

Atmosphere

Atmospheric conditions conducive to cell cultivation are maintained during irradiation in the device by Roos et al. and in ours. The device by Inkret et al. is used under room conditions. Because the dose rate obtained by their device is very high, the irradiation time is usually very short. Therefore, the critical atmospheric conditions conducive to cell cultivation are not required in their device.

It is generally concluded that a well balanced device useful for cell irradiation is obtained in the present work.

REFERENCES


