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A compact biological cell irradiation system with a Van de Graaff accelerator

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ABSTRACT

This report describes the development of a compact microcell irradiation system in the 3 MV KN Van de Graaff accelerator in the Accelerator Laboratory of National Tsing Hua University (NTHU). The main feature of this system is backscattering, a technique whereby a 100 nm gold scattering foil is placed in the center of a scattering chamber, instead of a 90° bending magnet. The incident particles produced by the accelerator bombard the scattering foil and scatter isotropically. A set of micro pinholes was installed above the scattering foil and directed the 90° scattering particles to irradiate the cell target. This innovation simplifies the system design and massively reduces the cost and space requirements for system construction.

1. Introduction

As radio-oncology progresses, developing an ion microbeam cell irradiation system for biological cells has gained importance. It is able to allow precise meter radiation dosage to individual cells, to increase the precision of radiation delivery, and to select individual cells or regions of tissue [1]. It offers a unique tool for studying DNA damage, and the bystander effect. In the past years, a number of laboratories have begun to construct micro-irradiation systems for radiobiological research [2,3]. They used either a set of complex micro-collimators or a series of quadrupole magnets to confine the beam spot size within the micrometer scale. Although both of these methods have been successfully applied in cell micro-irradiation experiments, constructing a microbeam system has limitations. The two most important limitations are its complex design and the costly equipment. For this report, we established a scattering chamber with a set of pinholes for micro-irradiation purposes. This system is constructed with the 3 MV KN Van de Graaff accelerator in the Accelerator Laboratory of National Tsing Hua University (NTHU).

2. Irradiation system design

Fig. 1 is a schematic diagram of the scattering chamber. Its main feature is that it uses a backscattering technique instead of a bending magnet that changes beam direction from horizontal to vertical. Backscattering theory and the numerical calculation are similar to those of our previous report [4]. However, the chamber size is much smaller for shrinking the distance between the scattering chamber and the cell-supporting chamber. A 100 nm gold scattering foil was placed at the center of the scattering chamber, and tilted at a 45° angle. A long carbon canal was placed in front of the scattering foil to confine the angle of the incident beam within 2°. To suppress the bremsstrahlung radiation, the inner wall of the scattering chamber was shielded with carbon sheets, and a beam absorption chamber was connected with the scattering chamber to absorb forward-moving particles. When the particles produced from accelerator were incident to the scattering chamber, they collided with the foil atoms and scattered isotropically. An exit aperture was opened immediately above the scattering foil to give the 90° scattering particles access to air for cell irradiation. Accurately measuring the number of irradiating particles on a cell is crucial [5,6]. In this system, the number of irradiating particles can be monitored by measuring the number of incident particles on the scattering foil.

3. Cell-supporting chamber and culture dish

A cell-supporting chamber, as Fig. 1 shows, was constructed on the exit aperture to provide a stress-free environment for the cell sample. In this chamber, the temperature was kept at 37 °C, and a 5% CO_2 mixed air flow was supplied continuously. The chamber

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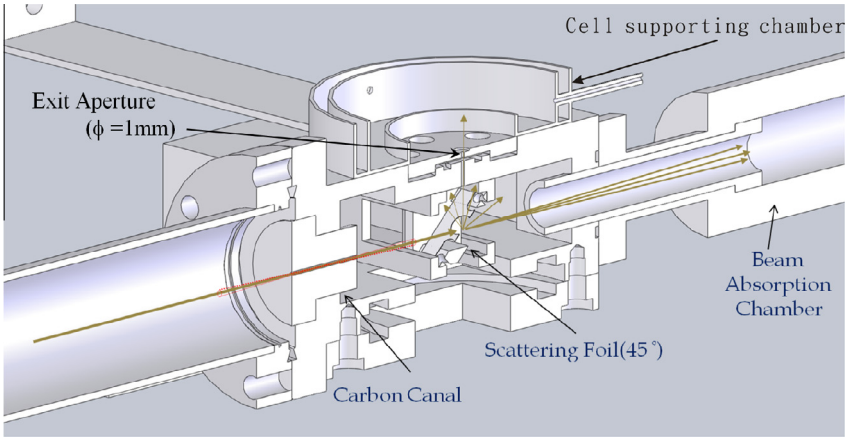

Fig. 1. The schematic diagram of the irradiation chamber.

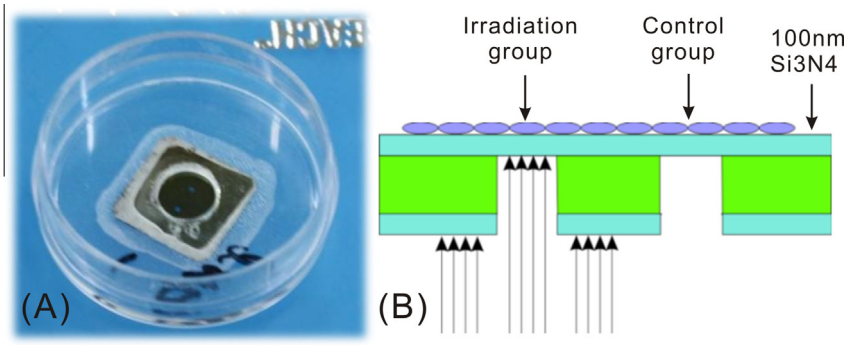

Fig. 2. The homemade cell culture dish. (A) Photo of the cell culture dish. An 8 mm width hole was opened at the bottom, and a sheet of a silicon nitride membrane chip was attached to it. (B) The schematic diagram of the silicon nitride membrane chip. This chip contains a layer of a 100 nm silicon nitride membrane, and two 1×1 mm^2 windows were opened on it.

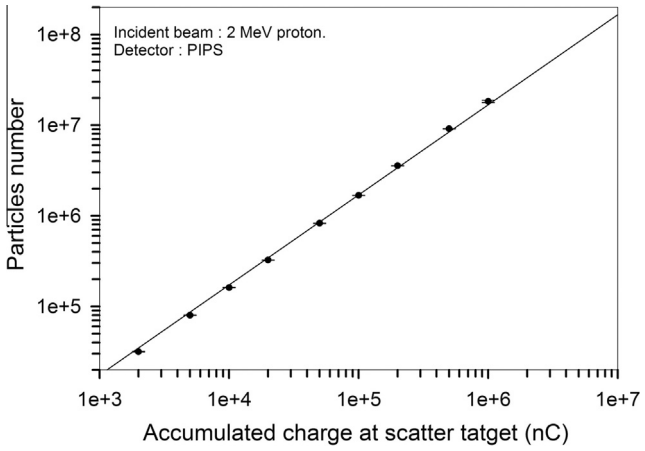

Fig. 3. The relationship between the outgoing particle number and the accumulated charge on the scattering foil. The solid line is the regression line.

was connected with an automatic three-axial high-precision stepper motor to control the cell position automatically and precisely. At the bottom of the supporting chamber, a 3.5 cm hole was opened at the center to mount a homemade culture dish. Fig. 2 shows a photo of the homemade cell culture dish. The base of the culture dish was a piece of silicon wafer. This wafer contained a 100 nm silicon nitride membrane layer, and two 1×1 mm^2 windows were opened on it, as shown in Fig. 2(B). These two windows divided the cell samples into an irradiation group and a control group, and the 5 mm gap prevented any interaction between them. The advantage of using the silicon nitride membrane-based culture dish is not only that it has the lowest energy loss, but also that it has the best cell adhesive ability [7].

4. Micro pinhole collimator

To extend the application of the irradiation system from broad beam irradiation to micro-irradiation, a micro-pinhole collimator was designed and constructed to shrink the spot size from a millimeter to micrometer scale. The micro-pinhole collimator was fabricated using the inductively coupled plasma reactive ion etching (ICP-RIE) technique to etch the silicon wafer. The ICR-RIE technique is commonly used in microelectromechanical systems (MEMS) processes to fabricate a high aspect ratio structure on silicon. It can achieve the requirements for a high etch rate and deep anisotropic silicon etching, and also maintains device dimensions from the mask to the base of the etched structures [8].

The range of 3 MeV proton particles in silicon is 93.3 μm and 48.4 μm for 2 MeV proton particles. To avoid multi-scattering in a micro-pinhole, which causes the irradiating spot size to increase, the thickness and diameter of the pinholes were designed in 100 μm and 5 μm, respectively. In this study, SF_6/O_2 and C_4F_8 were used as etch and passivation gasses, respectively, and the flow rates were 130/13 standard-state cubic centimeter per minute

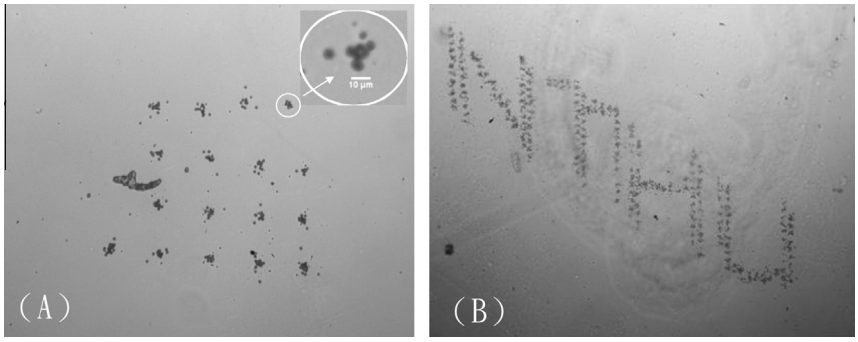

Fig. 4. The result of an automatic micro irradiation test. (A) A 4 × 4 array of 2.0 MeV particle tracks. In this array, 80% of the exiting particles were distributed within 10 μm in diameter. (B) The inscription of the initials of National Tsing Hua University (NTHU).

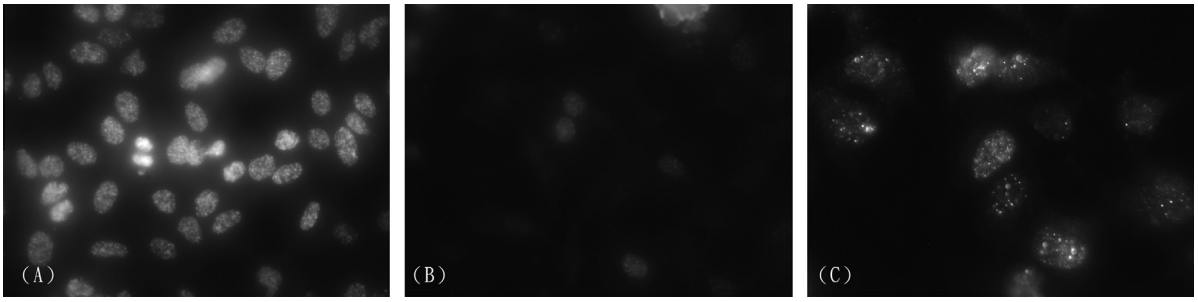

Fig. 5. The photon background test result. (A) The γ-H2AX proteins induced by proton irradiation. (B) The control group (without irradiation) shows no γ-H2AX signals. (C) The positive control group shows the γ-H2AX proteins that were also induced by X-ray irradiation.

(sccm) and 85 sccm. The RF power was 800 W. An aspect ratio of 1:20 in the micro pinhole with good sidewall verticality could be achieved. Two dies were aligned by laser and bonded with each other as a double-layer microcollimator (thickness 200 μm with a 5 μm diameter). This double-layer collimator could be placed on top of the beam aperture so that the irradiating spot size could be adjusted directly.

5. System performance test results

The system performance test was performed using a 2 MeV proton beam. A PIPS detector was mounted on the exit aperture, which has the same position as the cell-supporting chamber, to measure the energy spread and the number of outgoing particles. The measurement results indicated that the mean energy of the outgoing particles was 1.966 MeV, and the full width at half maximum (FWHM) was 30 keV. In the SRIM simulation calculation, the energy loss of 1.966 MeV protons in 100 nm Si_3N_4 was 5 keV [9]. Therefore, the energy losses of the proton beam before reaching the cell sample could be neglected when using the Si_3N_4-based culture dish in this study. Fig. 3 shows the relationship between the number of outgoing particles and the accumulated charge on the scattering foil. The results demonstrated that a good linear agreement exists between the particle number and the accumulated charge. Therefore, the number of outgoing particles could be easily measured by monitoring the scattering foil current. Fig. 4 shows the result of the automatic micro-moving irradiation test. In this test, a CR-39 track detector was placed at the bottom of the culture dish to register the particle hitting position. Fig. 4A shows that 80% of the outgoing particles were distributed within 10 μm in diameter, and Fig. 4B shows the CR-39 inscribed with the initials of National Tsing Hua University (NTHU).

6. Cell irradiation experiment

In the cell irradiation experiment, we tested the ability of this system to inflict radiation damage. First, we applied a 2 MeV alpha beam to irradiate HeLa cell to investigate the morphology change of cells. After irradiation, the cell samples were continuously incubated and observed for one week. After 48 h of delay, a giant cell formation could be easily observed in the irradiated group. The control group showed no abnormal signs.

To demonstrate that a significant photon background, which was produced by an energetic particle collision with the chamber wall, did not exist, cell samples were irradiated with 2.0 MeV protons in a dose range of 0.1–0.9 Gy. Other cell samples were exposed to a 150 kVp X-ray tube in an exposure range of 0.28–0.84 Gy as the positive control. After irradiation (by protons or X-rays), the immunofluorescence staining method was used to evaluate the accumulation of γ-H2AX histon proteins. The result is shown in Fig. 5. The γ-H2AX proteins could be induced by proton irradiation, and there were no γ-H2AX signals in the control group. In the positive control, the γ-H2AX proteins could be also induced by X-ray irradiation. This demonstrates that the influence of the photon background around the scattering chamber was non-significant.

7. Summary

The results of the performance test show that the system was proved for cell irradiation application. In addition to space and cost being largely reduced by removing the 90° magnet, the number of outgoing particles could be easily obtained, and the irradiating spot size could be adjusted from a millimeter to micrometer scale by changing the pinhole collimator.

Acknowledgments

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