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An integrated on-line irradiation and *in situ* live cell imaging system

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ABSTRACT

Ionizing radiation poses a threat to genome integrity by introducing DNA damages, particularly DNA double-strand breaks (DSB) in cells. Understanding how cells react to DSB and maintain genome integrity is of major importance, since increasing evidences indicate the links of DSB with genome instability and cancer predispositions. However, tracking the dynamics of DNA damages and repair response to ionizing radiation in individual cell is difficult. Here we describe the development of an on-line irradiation and *in situ* live cell imaging system based on isotopic sources at Institute of Heavy Ion Physics, Peking University. The system was designed to irradiate cells and *in situ* observe the cellular responses to ionizing radiation in real time. On-line irradiation was achieved by mounting a metal framework that hold an isotopic γ source above the cell culture dish for γ irradiation; or by integrating an isotopic α source to an objective lens under the specialized cell culture dish for α irradiation. Live cell imaging was performed on a confocal microscope with an environmental chamber installed on the microscope stage. Culture conditions in the environment chamber such as CO_2, O_2 concentration as well as temperature are adjustable, which further extends the capacity of the system and allows more flexible experimental design. We demonstrate the use of this system by tracking the DSB foci formation and disappearance in individual cells after exposure to irradiation. On-line irradiation together with *in situ* live cell imaging in adjustable culture conditions, the system overall provides a powerful tool for investigation of cellular and subcellular response to ionizing radiation under different physiological conditions such as hyperthermia or hypoxia.

1. Introduction

DNA damage is induced in cells as a consequence of exposure to ionizing radiation. Among the various kinds of DNA damages, DNA double strand break (DSB) is believed to be most severe since it is difficult to repair and, if misrepaired or left unrepaired, may lead to mutation or genomic instability. Cells have evolved different ways of antagonizing DNA damage such as cell cycle arrest, DNA repair and apoptosis to maintain genome integrity [1–3]. Cellular response to ionizing radiation is of fundamental importance in radiobiology since we are constantly exposed to environmental or medical radiation. However, the early inchoate cellular responses, especially the dynamic process of DSB generation and repair, are seldom revealed in conventional radiation experiments due to the physical separation between irradiation facility and microscope.

On-line irradiation provides an ideal approach to investigate this issue because it requires no transfer of cells from one place to another after irradiation. Combined with live cell imaging technics, on-line irradiation is advantageous over traditional off-line irradiation and fixed cell experiment, mainly in that targeted subcellular irradiation is feasible and the dynamic response of an individual cell after ionizing radiation is presented. Taking advantages of on-line irradiation, problems concerning low dose radiation effects, spatiotemporal organization of DNA repair factors and chromatin movement at damaged site could be directly addressed [4–6].

One way of realizing on-line irradiation is to construct a customized microscope at an accelerator beam line end-station. This kind of facilities has been proposed and constructed on many accelerators and achieved great success. Moreover, it could be further developed as microbeam facility by adding a series of focusing, collimating and controlling devices to achieve localized and single-ion irradiation. Nowadays, there are more than 30 microbeam facilities in operation or under construction worldwide [7]. However, the complex accelerator beam line based on-line irradiation facility costs much and suffers from beam time limitation.

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Radioisotopic source based on-line irradiation is another way of choice. One of the pioneering works was done in 1958 by T.R. Munro who innovatively delivered α particles to a selected part of cells using polonium deposited micro-needle [8]. It is gaining popularity for its small physical dimensions and applicability in general laboratories. Jan Stap and Przemek M. Krawczyk devised an on-line irradiation device by mounting a small Americium α source box on a mechanical arm, and described a procedure for induction of markedly linear arrays of DSB in adherent eukaryotic cells [9]. Steeb and colleagues developed a microirradiation system by mounting a Nickel-63 β-ray microirradiator on a micromanipulator installed on the microscope stage [10]. Using this system, they found a subset of DSB foci that appear and disappear rapidly before a plateau level is reached 30 min post-irradiation. However, problems remain for these isotope based facilities primarily consist in that the experimental procedure is complicated and cell culture condition could not be well controlled.

Here we describe a novel design of radioisotopic source based on-line irradiation system. This system is capable of providing two irradiation modalities, γ and α irradiation. For γ irradiation, we mount a metal framework in the environmental chamber just above the cell culture dish to support an isotopic γ source. For α irradiation, we integrate an isotopic α source to an objective lens and irradiate cells from under the specialized dish. By rotating the objective stage, we could easily switch between irradiation mode and observing mode. Besides irradiation, the system is equipped with a heated environmental chamber along with a CO_2 module and an O_2 module for cell incubation. The functionality and stability of the whole system was tested. Human fibroblast HT1080 cells stably transfected with EGFP tagged 53BP1 were used to track individual DSB foci immediately after ionizing radiation. Initial study suggests a distinct rate of foci formation after exposure to different radiation type.

2. Materials and methods

2.1. Microscope

The system was constructed on an inverted confocal microscope Carl Zeiss LSM700. The confocal microscope was equipped with a heated, humidified environmental chamber Heating Insert P S1 along with a CO_2 module and an O_2 module for culture condition adjustment and control.

2.2. Isotopic γ source and irradiation

The isotopic γ source we use is an ^{241}Am low-energy γ source. When ^{241}Am decays, it emits an α particle along with a 59.5 keV γ photon. Radioactive ^{241}Am was electroplated on a stainless steel substrate with a diameter of 15 mm and a thickness of 1.8 mm and covered with 0.1 mm AuPd foil to prevent leakage and block α particles. For safety consideration, we stick the source to a 7 mm thick steel shield to attenuate γ-rays. The activity of the low-energy γ source is 1.2 mCi and the estimated dose rate in our irradiation setup is about 0.1 Gy/min.

To irradiate cell using this γ source, a metal framework is devised to support the source above the culture dish and accommodate within the environmental chamber. The setup of the metal framework and the γ source is shown in Fig. 1. We load the γ source on the metal framework and put it into the environmental chamber to irradiate cells. After a specified dose is reached, we take out the metal framework for post-irradiation observations. Images could be acquired before, during and post exposure since radiation is delivered from above the dish, which did not interfere with the light path below. When manipulating this γ source device, radio-protective gloves, glasses and clothes are prerequisite for personal safety.

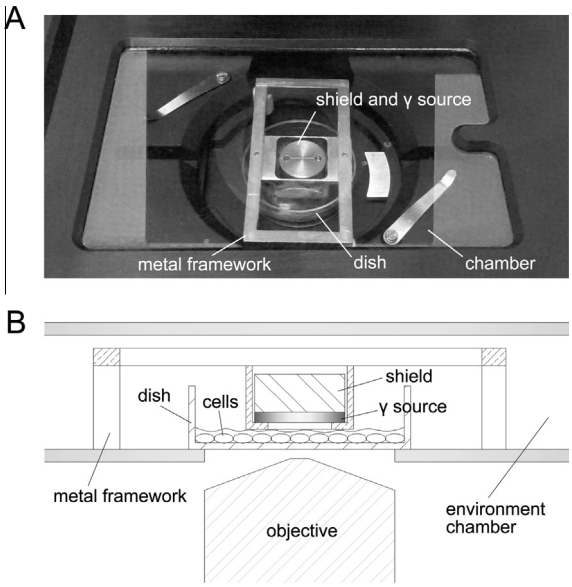

Fig. 1. Setup of the γ irradiation device. (A) Close-up view of the γ irradiation device. A metal framework was set in the incubator across the cell culture dish to support the γ source. (B) Schematic drawing of γ irradiation device.

2.3. Isotopic α source and irradiation

The isotopic α source we use is an ^{241}Am α source. Radioactive ^{241}Am was electroplated on a silver substrate with a diameter of 14.8 mm and a thickness of 0.2 mm and then enclosed with 1.5 μm AuPd foil to prevent surface contact contamination. The enclosed α source has a radioactive region about 10 mm in diameter and an activity of about 1.4×10^5 Bq. It is estimated that there are 2–3 α particles traverse cell nucleus per minute. ^{241}Am emits a monoenergetic 5.4 MeV α particle when it decays. The 5.4 MeV α particle has a linear energy transfer of about 0.4 MeV/μm in AuPd alloy. Trajectory length of the α particle in the 1.5 μm AuPd foil is about 1.5–3 μm, correspond to perpendicular (90°) traversal and 30° traversal. After passing through the AuPd foil, α particle energy drops to about 4.2–4.8 MeV in the 30–90° range, which is still sufficient to penetrate the cell nucleus.

The setup of α irradiation is shown in Fig. 2A. We integrate the α source to the microscope by sticking it to the top surface of a 20× objective lens (Fig. 2B). If we want to irradiate the cells, we just rotate the objective lens stage making this α source lens at central position. In case we use an oil immersion objective for pre-irradiation imaging, we set the objective stage to load position and the objective will separate from the dish bottom. Next we use a piece of oil blotting paper scrape against the bottom gently to absorb the oil. Repeat this for one or two times until the oil is removed completely. Then we rotate the objective stage making the 20× objective and α source at central position and raise the stage up for irradiation. After irradiation, we rotate the objective lens stage and put other objectives at central position for real time observation (Fig. 2C and D). These operations could be done by computer software. The thickness of the α source is added to the length of the 20× objective in the system parameters to avoid crashing into the cell dish. The low activity of the enclosed α source and the limited range of α particle in air allow handling the source with just radio-protective gloves and no other special precautions.

According to SRIM calculation, the 4.2–4.8 MeV α particle has a range of about 28–34 μm in glass, which is far less than the