Nuclear Instruments and Methods in Physics Research B 269 (2011) 3120–3124

Contents lists available at ScienceDirect

Nuclear Instruments and Methods in Physics Research B

journal homepage: www.elsevier.com/locate/nimb

In vitro irradiation station for broad beam radiobiological experiments

A.-C. Wéra [a,*], H. Riquier [b], A.-C. Heuskin [a], C. Michiels [b], S. Lucas [a]

[a] NAmur Research Institute for LIfe Sciences (NARILIS), Research Centre for the Physics of Matter and Radiation (PMR), University of Namur-FUNDP, Belgium
[b] NAmur Research Institute for LIfe Sciences (NARILIS), Unité de Recherche de Biologie Cellulaire (URBC), University of Namur-FUNDP, Rue de Bruxelles, 61, B-5000 Namur, Belgium

ARTICLE INFO

Article history:
Available online 28 April 2011

Keywords:
Alpha particles
Proton
Carbon ions
Survival fraction
A549 lung cancer cell
Radiobiology

ABSTRACT

The study of the interaction of charged particles with living matter is of prime importance to the fields of radiotherapy, radioprotection and space radiobiology. Particle accelerators and their associated equipment are proven to be helpful tools in performing basic science in all these fields. Indeed, they can accelerate virtually any ions to a given energy and flux and let them interact with living matter either *in vivo* or *in vitro*. In this context, the University of Namur has developed a broad beam *in vitro* irradiation station for use in radiobiological experiments. Cells are handled in GLP conditions and can be irradiated at various fluxes with ions ranging from hydrogen to carbon. The station is mounted on a 2 MV tandem accelerator, and the energy range can be set up in the linear energy transfer (LET) ranges that are useful for radiobiological experiments. This paper describes the current status of the hardware that has been developed, and presents results related to its performance in term of dose-rate, energy range and beam uniformity for protons, alpha particles and carbon ions. The results of clonogenic assays of A549 lung adenocarcinoma cells irradiated with protons and alpha particles are also presented and compared with literature.

1. Introduction

Since 1946, with the proposal of Wilson [1], the use of charged particles in radiobiology has encountered an increasing interest. The development of cancer treatments using particle accelerators has continued to this day. In 2005, Amaldi found that more than 40% of the 17,500 existing particle accelerators were used for radiotherapy, with about 40,000 and 2200 patients treated with proton beams and carbon ions, respectively [2]. At the same time, fundamental research performed with particle accelerators was yielding useful information for medical applications as well as for radioprotection and space radiobiology [3–8]. The strength of a particle accelerator lies in its wide range of available ions for which energies and flux can be greatly varied. There are two configurations: microbeams and broad beams. On the one hand, micro-/nano-beams allow the irradiation of a selected cell, or a portion of a cell, with a precise number of ions [9–16]. However, microbeam installation requires tedious developments for cell recognition, alignment and beam scanning. On the other hand, broad beams are easier to implement [17–22]. Furthermore, this system also allows simultaneous treatment of thousands of cells within minutes. Therefore, with broad beams, additional stresses like temperature effects or absence of culture medium during irradiation can be minimized. Nevertheless, as is the case for patient irradiation, not all the cells can be targeted because of the Poisson distribution of the beam.

In 2007, the LARN laboratory at the University of Namur started to develop an inexpensive broad beam *in vitro* irradiation station. First results were published in 2008 regarding proton beam characterization [23]. In this paper, we describe the hardware development for our irradiation station, as well as present results related to its performance in term of energy straggling, beam uniformity and monitoring for proton, alpha and carbon ions beams. The irradiation station was used to irradiate A549 non-small-cell lung cancer adenocarcinoma cells (NSCLC) with proton and alpha beams at 1 Gy/min. The survival fraction curves assessed by conventional clonogenic assays are compared to literature. Radiosensitivity parameters, as well as the inactivation cross-section, are calculated.

2. Material and methods

2.1. Irradiation facility

The irradiation station is placed on a 2 MV tandem accelerator (High Voltage Engineering Europa) available at the LARN laboratory. Thanks to its dual source, particles from hydrogen to uranium can be accelerated with a terminal voltage adjustable from 0.15 to 2 MV. The irradiation station is fixed at the end of the 10° exit port of the switching magnet. The irradiation field is set up by defocus-

* Corresponding author. Tel.: +32 81725479; fax: +32 81725474.
E-mail addresses: anne-catharine.wera@fundp.ac.be (A.-C. Wéra), helene.riquier@fundp.ac.be (H. Riquier), anne-catherine.heuskin@fundp.ac.be (A.-C. Heuskin), carine.michiels@fundp.ac.be (C. Michiels), stephane.lucas@fundp.ac.be (S. Lucas).

0168-583X/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.nimb.2011.04.104

ing the beam with the help of electrostatic and magnetic optical elements mounted along the beam line. The irradiation station is schematically presented in Fig. 1. It consists of a vacuum chamber (2) in which a removable CCD camera is mounted with a BC400 scintillator (4). The camera is used to tune the beam at a high current. The beam is extracted to air through an exit window (6) made of a 8 μm Kapton foil maintained between two stainless steel cylinders (see insert of Fig. 1). A collimated PIPS (passivated implanted planar silicon) detector is placed just before the irradiation head (5). This detector intercepts the left side of the beam and is used as a dose-rate monitor during the irradiation. Another PIPS detector (7) is fixed on an XY table, which is activated by a step motor (8) and which is placed just after the exit window. The exposed surface of this detector was limited with a collimator (Ø 510 ± 2 μm). This is used to adjust the dose-rate, and to assess the stability and the homogeneity of the broad beam.

2.2. Dosimetry

The dose-rate is given by:

$$\dot{D}\,(\mathrm{Gy/s}) = 1.6 \times 10^{-9}\,\frac{\mathrm{LET}\,(\mathrm{keV/\mu m}) \times \Phi(\mathrm{part/cm^2\ s})}{\rho(\mathrm{g/cm^3})} \qquad (1)$$

where LET is the linear energy transfer, Φ is the flux and ρ is the cell density. Therefore, to perform radiobiological studies with a broad beam, the LET and the flux need to be known and constant over the entire surface covered by the biological cells and throughout the irradiation.

As the LET is related to the beam energy, a precise energy calibration of the accelerator is necessary. The experimental detail and results of the calibration were presented in [24]. The LET value, calculated by the SRIM program [25], can be scanned from 10 to 50 keV/μm for proton and from 90 to 200 keV/μm for alpha particles with energies ranging from 4 MeV to 800 keV and from 6 to 3 MeV for proton and alpha particles, respectively. For carbon ions, the Kapton exit foil is replaced by a 3 μm Mylar foil, and LET from 650 to 900 keV/μm are achievable.

The flux can be adapted to obtain a large range of dose-rates (typically from 0.1 to 10 Gy/min). Its stability is checked over time and over the irradiation surface. The beam uniformity is checked by moving the PIPS millimeter by millimeter to obtain a 1 × 1 cm map. The results presented in this paper were obtained for a fixed dose-rate of 1 Gy/min for all ions ($^{1}H^{+}$, $^{4}He^{2+}$, $^{12}C^{4+}$).

2.3. Cell culture and irradiation

Human A549 NSCLC cells were grown in MEM (minimum essential medium) (Invitrogen, UK) containing 10% (v:v) fetal calf serum (FCS) (Invitrogen, UK). Twenty-four hours before irradiation, 100,000 cells were seeded as a 35 μl drop at the centre of the Kapton foil (exit window) of pre-sterilized irradiation heads (see Fig. 1). These heads are then closed with a plastic cap to avoid dehydration and contamination and are placed in an incubator at 37 °C with 5% CO_2. Six hours after seeding, the drop is rinsed twice with phosphate-buffered saline pH 7.4 (10 mM phosphate, 0.9% NaCl) (PBS) to remove non-adherent cells. The irradiation heads are then filled with culture medium and replaced in the incubator. Just before the irradiation, the culture medium is replaced by independent CO_2 medium. After the irradiation, the CO_2 medium is poured off and the irradiation heads are rinsed with PBS. The plastic cap is then removed, and a sterile cotton swab is used to take away the cells that may have detached from the central drop and diffused outside the irradiated field. The plastic cap is replaced on the irradiation heads for another PBS wash. Cells are detached by using trypsin, then counted and seeded in 6-well plates (9.40 cm^2) at desired concentrations. Cells are also seeded in a separated dish at one concentration per dose and 2 h later the cells are fixed with paraformaldehyde (PFA) 4% for 10 min before three PBS washes. The number of cells attached to the dish is counted manually under an optical microscope to precisely know the number of cells seeded for each concentration and dose. Eleven days post irradiation, the number of visible colonies (containing more than 50 cells) is counted after staining with crystal violet in 2% ethanol. The plating efficiency (PE) is determined for each dose and the survival fraction is calculated by evaluating the ratio of the PE for the irradiated cells to the PE for the control cells. At least three independent experiments are performed for each dose and the errors are evaluated as a standard deviation. Note that the control cells underwent exactly the same steps as the irradiated cells. In this

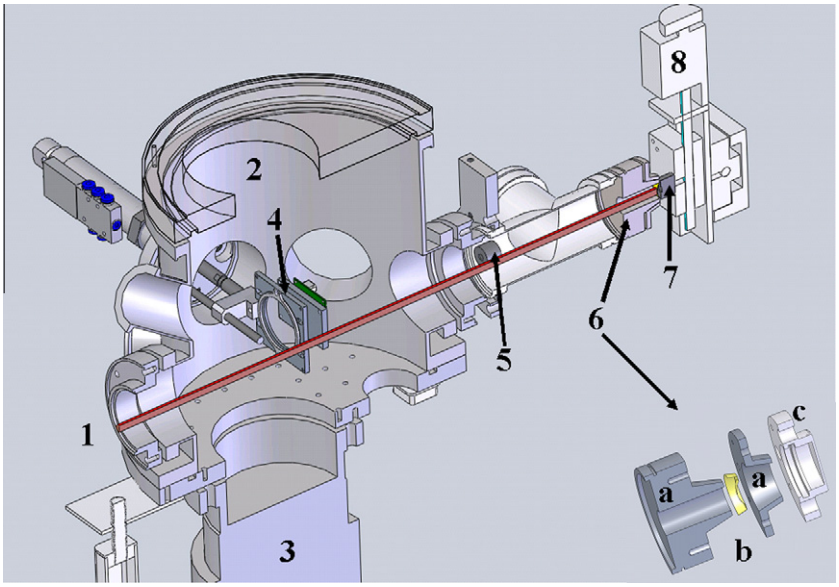

Fig. 1. *In vitro* irradiation station for a broad beam. (1) ^{+}H, ^{2+}He, ^{4+}C broad beam; (2) vacuum chamber; (3) pumping system; (4) BC400 scintillator + CCD camera fixed on a pneumatic jack; (5) PIPS detector placed on the left side of the beam for the dose-rate monitoring; (6) irradiation head; (7) movable PIPS detector; (8) XY motor. Detail of the irradiation head: (a) stainless steel cylinder; (b) 8 μm Kapton foil; (c) plastic cap.