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The Gray Cancer Institute X-ray microprobe and its radiobiological applications

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Abstract. Radiation microbeams represent a unique and powerful tool to study and quantify the effects of precise doses of radiation delivered with micron precision to selected biological samples. The Gray Cancer Institute has developed two independent but complementary micro-irradiation facilities, specifically developed for the targeted irradiation of cells and structured tissues; a charged-particle microbeam that uses collimated protons or helium ions and an ultra-soft X-ray microprobe. The ultra-soft X-ray facility employs a focused electron bombardments source to produce a near monochromatic C_K X-ray beam. Highly efficient zone plates optimised for the appropriate wavelength are used to focus the characteristic X-rays into a sub-micron spot. The facility is also equipped with a three-axis micro-positioning stage, an epi-fluorescent UV microscope with intensified CCD camera coupled to a fast PC for a automatic, fast and accurate samples recognition and alignment with the probe. Recent experiments have been directed to investigate the bystander effect by irradiating only one cell within a population of V79 cells that are subsequently individually revisited for colony formation. A clear bystander effect has been detected (~ 10 % reduction in survival) when a single cell has been irradiated. The effect is triggered by very low doses (~ 100 mGy) and it is largely dose independent.

1. INTRODUCTION

Although the advantage of accurate individual irradiation of samples has always been recognised in radiobiology, only the fast technological improvements of the last decades has made it possible the designing and realisation of complex facilities commonly called microbeams/microprobes. Microbeams are facilities designed to individually irradiated a large number of cells, or part of them, with a very fine beam of ionising radiation and subsequently assess the extent of the damage produced. In the recent years an increasing number of microbeams facilities have been devolved to radiobiological studies and the quality of the experiments performed underlines the fundamental role that these facilities may play. The Gray Cancer Institute has a well-established history and reputation in developing state of the art single cell micro-irradiation facilities for precise and accurate radiobiological studies. In the recent years, efforts have been concentrated in the development of a second facility to complement our existing charged-particle microbeam [1].

1.1 Facility description

The ultrasoft X-ray microprobe is based on a laboratory bench electron bombardment source to produce a near monochromatic X-ray beam [2] emerging vertically from the source. Electrons, produced by a heated tungsten filament are accelerated up to 10 keV and focused by an electromagnetic lens into a solid target. In this way, characteristic X-rays are produced together with a continuous bremsstrahlung background. The high-energy component of the bremsstrahlung is eliminated by forcing the produced photons to be reflected at a shallow angle on a polished silica (SiO_2) mirror before emerging the source. Thin films are used to eliminate the eventually low-energy

component. With this process a near monochromatic X-ray beam is obtained [3] at the expenses of a lower characteristic X-ray output as shown in figure 1a. The production efficiency for characteristic X-rays has also been investigated in order to optimise the source performances. When the yield of characteristic photons produced is plotted against the energy of the accelerated electrons, a parabolic curve is obtained (i.e. figure 1b obtained using a graphite target). The sharp increase in characteristic X-ray production is due to higher energy deposited into the target by the bombarding electrons. However, as the electrons penetrate deeper and deeper into the target, characteristic X-rays are produced deeper inside the target and self-absorption by the target itself play a major role determining a decrease in X-ray production. Although the best electron energies for the target considered (C, Al and Ti) are higher than 10 keV, the amount of power that can be dissipate into the target impose some limits on the electron energy that can be used with the actual source configuration [3]. The characteristic X-ray production as function of the target current (i.e. number of electron hitting the target) is extremely linear as expected and it is used as parameter to monitor the X-ray output.

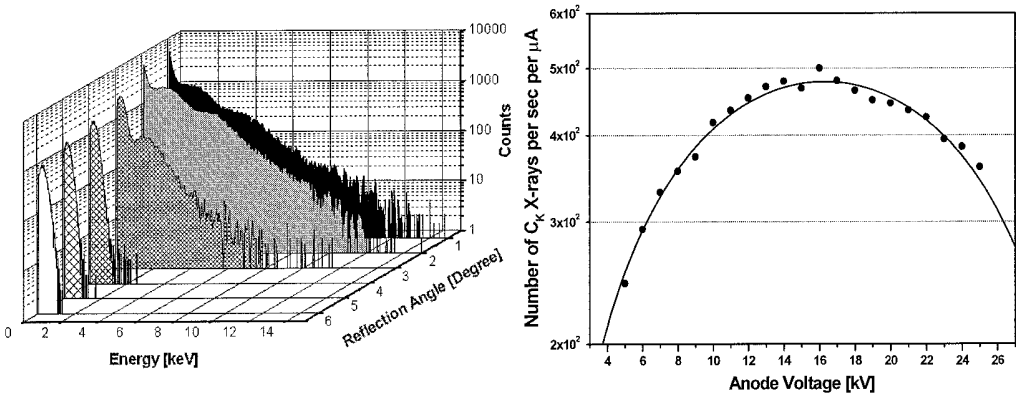

Figure 1. a) Energy spectra emerging from the X-ray source (graphite target) as a function of the reflection angle on the silica mirror. **b)** Yield of $C_{K\alpha}$ produced as a function of the energy of the striking electrons (anode voltage).

The near monochromatic X-ray beam is then focused into a sub-micron spot by using diffraction optics tools, i.e. zone plates optimised for the geometry and the wavelengths used. The zone plate and the order-selecting aperture (a 12.5 μm pinhole) are aligned in order to let only the first order focusing radiation reaching the samples by using a specially designed arrangement. Magnetic strips keep the elements in the aligned position while their distance can be precisely adjusted. A three-axis micro-positioning stage with a 0.25 nm resolution is placed right above the source. The stage is initially used to align the optical elements and subsequently to support the sample for their alignment with the radiation probe. Finally, an infinite optic microscope coupled to a CCD camera and an intensifier is position above the stage with its objective looking down toward the probe. A PC controls the whole system in order to perform fast and accurate repetitive routines, i.e. image analysis for cell finding and micro-positioning for cell irradiation.

1.2 X-ray microprobe characteristics

The microprobe was initially designed for using carbon K X-rays (278 eV) but it has recently been updated to aluminium K_α X-rays (1.45 keV) and work is in progress to use also titanium K_α X-rays (4.5 keV). As the dose distribution and the pattern of ionisations produced inside the biological samples strongly depends on the energy of the X-rays [4], this range of energies available will make the microprobe an even more powerful tool for investigating the effect of ionising radiation. In particular, following irradiation with a C_K X-ray beam, most of the energy will be deposited within the first few microns inside a cell producing clusters of ionisation of < 7nm range. Contrary, irradiation

with an Al_K X-ray beam will produce a more uniform energy distribution with ionisations spread over a 70 nm range. Finally, using Ti_K, it will be possible to irradiated biological targets several hundreds of microns inside a well-organised 3D cell structures.

The performances of the X-ray microprobe are mainly related to the wavelength of the radiation used, as lower energy photons are easier to produce, separate from the bremsstrahlung component and focus. The table 1 summarises some of the microprobe performances as a function of the X-rays used.

Table 1. X-ray microprobe performances.

X-rays	ZP used	Beam Purity [%]	Beam size [μm]	Output [hν/sec]
C_K	Si_3N_4 – 200 μm R	98	< 0.5	250 10^3
Al_K	Ge – 300 μm R	95	< 0.5	4 10^3
Ti_K^*	W – 100 μm R	90	< 0.5	100

* Expected values from preliminary measurements and simulations.

Beam size measurements are performed by monitoring the X-ray output with a detector (home designed proportional chamber of C_K X-rays and a solid-state detector (XR-100CR AmpteK) for Al_K and Ti_K X-rays) while scanning a knife-edge mask across the X-ray focal plane. The knife-edge mask is coupled to the micro-positioning stage for an accurate controlled scanning. The size of the X-ray focus can be determined by analysing the changes in the dose rate as a function of the mask movement. The position of the mask as it cuts off the X-ray beam, indicates also the precise co-ordinate of the X-ray probe relative to the OSA.

2. BIOLOGICAL DATA

The ultrasoft X-ray microprobe has already been used to investigate critical radiobiological phenomena by a number of years. Its high sensitivity and flexibility in irradiating only selected cells by carrying out experiments on a single cell basis, makes the microprobe an ideal tool to study the bystander effect. A number of traditional experiments have reported the possibility that radiation damage is transmitted from the irradiated cells to their un-irradiated neighbours [5]. Nevertheless, a better understanding of the mechanisms involved and more accurate analysis are required in order to formulate an adequate model that would consider the bystander effect in the whole radiobiology scenario.

2.1 Experimental procedure

Using the soft X-ray microprobe, it has been possible to accurately investigate the clonogenic potentials of V79 cells exposed to a focused beam of carbon K X-rays. For this experiments, single cells are seeded on a think Mylar base film (0.9 μm) at a quite low concentration (~ 10 cells / mm^2). The seeding protocol [6] has been optimised in order to be able to correlate every eventual future colony with its mother cell. Once the cells are attached (4 h), their nuclei are stained with a fluorescent DNA binding (Hoechst 33258) at a non-toxic concentration (1 μM for 1 h). In this way, cell nuclei can be automatically localised with an image analysis algorithm during an UV scan of the cell dish. The co-ordinates of all cells are recorded and the selected cells are then irradiated. After an incubation period of 3 days, necessary for the surviving cells to form a healthy clone (> 50 cells), the co-ordinates are revisited and the presence of clones assessed. Control dishes are exposed to the same procedures but no irradiation is delivered to the cells.

2.2 Bystander effect

Following the irradiation of all cells seeded in the dish, a clear linear quadratic dose response is obtained (figure 2), in good agreement with previous data [4] obtained with traditional clonogenic techniques. On contrary, a significant bystander effect is detected when only a single cell per dish is irradiated. The extent of the effect is a statistically significant decrease in survival (~10 %). The effect is initially dose-dependent (< 0.2 Gy), where no statistical difference is observed between the all cell and the single cell irradiation, to then reach a plateau (up to 2 Gy).

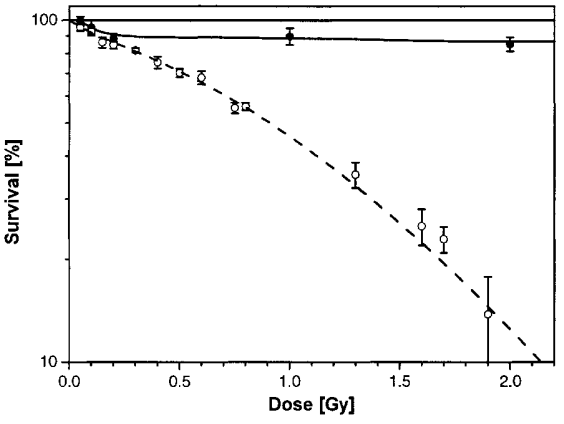

Figure 2. V79 survival measurements following the irradiation of all cells present in the dish (O) or of a single sample (●).

Experiments are still in progress to investigate the relevance of the cell cycle and determine the presence of factors that may alter the extent of the bystander effect.

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References

[1] – M. Folkard, Vojnovic B., Schettino G., Forsberg M., Bowey G., Prise K.M., Michael B.D., Michette A.G., Pfauntsch S.J., Nuclear Instruments and Methods in Physics Research B, **130**, 270-274 (1997)
[2] - M. Folkard, Schettino G., Vojnovic B., Gilchrist S., Michette A.G., Pfauntsch S.J., Prise K.M., Michael B.D., Radiation Research, **156**, 796-804 (2001)
[3] – G. Schettino, Folkard M., Vojnovic B., Michette A.G., Stekel D., Pfauntsch S.J., Prise K.M., Michael B.D., Radiation Research, **153**, 223-225 (2000)
[4] - M. R. Raju, Carpenter S.G., Chmielewski J.J., Schillaci M.E., Wilder M.E., Freyer J.P., Johnson N.F., Schor P.L., Serbing R.J., Goodhead D.T, Radiation Research, **110**, 396-412 (1987)
[5] - H. Nagasawa, Little J.B., Radiation Research, **152**, 552-557 (1999)
[6] - G. Schettino, Folkard M., Prise K.M., Vojnovic B., Bowey A.G., Michael B.D., Radiation Research, **156**, 526-534 (2001)