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The impact of microbeams in radiation biology

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Abstract

Cellular micro-irradiation is now recognised as a powerful technique for understanding how ionising radiation interacts with living cells and tissues. Charged-particle microbeams are uniquely capable of delivering single, or counted multiple particles to selected sub-cellular targets. This capability is particularly useful for studying the risks associated with environmental exposures to α-particle emitting isotopes (such as radon) where exposed cells within the body are unlikely to receive more than one particle traversal. Microbeam methods are also seen as highly appropriate for studying the so-called 'bystander effect' (where unirradiated cells respond to signals transmitted by irradiated neighbours). Using the Gray Laboratory microbeam, we have been able to demonstrate a significant increase in the levels of cell death and DNA damage in a population of cells after irradiating just a few cells within a population. Also, by targeting the cell cytoplasm, we have shown that intra-cellular signalling between the cytoplasm and nucleus can cause DNA damage, showing that direct DNA damage is not required to observe radiation induced effect in cells.

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1. Introduction

Until the last few years, there has been very little active research using microbeams of ionising radiation for radiobiological applications, even though charged-particle microprobes have been well established as an analytical tool in a number of other research areas for several decades. Interestingly however, one of the first uses for a charged-particle microbeam was in a radiobiological application; in the 1950s, Zirkle and Bloom developed and used a collimated, low-energy proton microbeam to study the fidelity of cell division following the irradiation of cells in metaphase [1]. The resurgence of interest in the use of microbeams in radiation biology is due in part to the substantial technological developments that have occurred in computing and electronic imaging over the past decade. Modern technology makes possible the design and construction of fast, automated micro-alignment systems that are necessary for many radiobiological applications. The initial impetus for the development of these facilities was

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the recognition that charged-particle microbeams are uniquely capable of delivering single, or counted multiple particles to individual cells, and therefore particularly useful for studying the risks associated with environmental exposures to α-particle emitting isotopes. It is also fortuitous that as the first of the modern radiobiological microbeams became operational, interest in research towards understanding the targets and signalling pathways required for various radiobiological responses increased significantly, and for which the involvement of microbeams was, and still is, highly appropriate. Consequently, there is now widespread interest amongst the radiobiological community in microbeam-type studies. This has prompted a number of research groups to develop, or adapt existing microbeams for radiobiological use.

Our own microbeam facility at the Gray Laboratory is fully operational, and has been in routine use for some years [2,3]. Similarly, the radiological research accelerator facility (RARAF) at Columbia University (New York) has been routinely operating a fully automated microbeam for irradiating cells since the 1990s [4]. A heavy-ion microbeam facility exists at the Japan Atomic Energy Research Institute (JAERI, Takasaki, Japan) and has been developed to micro-irradiate living organisms with 10 MeV/u ions from a cyclotron [5]. The system is fully developed and operational (including automated cell-finding procedures) but is available for radiobiological experiments only intermittently. A number of other radiobiological microbeam facilities worldwide are in various stages of development. Partly developed facilities exist at Texas A&M (relocation of a formerly operational microbeam [6,7]), GSI Darmstadt (adaptation of an existing heavy-ion microprobe [8]), CENBG Bordeaux (based on an existing light-ion microprobe [9]), PTB Braunschweig and MIT Boston. Microbeams that use ionising radiations other than charged-particles also exist. At the Gray Laboratory, we have developed a second single-cell micro-irradiation facility that uses X-ray diffraction optics to focus 278 eV X-rays to a sub-micron spot [10]. Low-energy X-rays have the advantage that they interact almost entirely through photoelectric absorption, such that scattering by the vacuum window and other intervening materials does not degrade the resolution.

Several significant microbeam-related radiobiological studies have recently been published, emanating largely from work using either the Gray Laboratory or the RARAF facility. Already, these studies are beginning to have an impact on our understanding of radiation risk, and on the pathways by which radiation damage can be transferred by both inter- and intra-cellular signalling mechanisms. The impact of microbeams in this field is expected to increase as more facilities become available.

2. Design of microbeams for irradiating cells

To develop a microbeam for a radiobiological application presents the designer with a unique set of problems, not least, the requirement for the cellular target to be in a humid environment at atmospheric pressure. There is also a considerable practical advantage in using a vertically-oriented beam, rather than the horizontal configuration common to microprobes used in analytical applications (despite this, the facilities under development at GSI Darmstadt and at CENBG Bordeaux will both utilise existing horizontal microprobes). There are two methods that can be used to achieve a micron-sized particle beam: collimation and focusing. While focusing is ultimately capable of the producing the finest beams, the benefits are easily lost by the requirement for a particle detector and for a vacuum window, both of which can scatter the beam (less so however when heavy-ions are used). Focusing can also produce much higher-dose rates, although intense beams are not necessary for radiobiological use. It is also the case that many conventional focusing systems may simply be too costly, or require too much space to be a viable option when adapting an existing accelerator for use as a microbeam. Consequently, all microbeams currently in use for cellular studies utilise collimation, rather than focusing (although the RARAF group are developing an electrostatic focusing system [11]). Clearly however, where facilities are

being developed around existing microprobes, the focusing systems already in operation will be used. Despite the less favourable spatial resolution of collimated systems, this method can have sufficient resolution for many radiobiological applications. The Gray Laboratory microbeam uses a 1 μm diameter bore glass capillary to vertically collimate protons, or helium-ions accelerated by a 4 MV Van de Graaff [3]. We have determined the targeting accuracy of our collimated facility using CR-39 track-etch plastic. Our measurements show that for protons, we can hit 90% of targets with an accuracy of ±2 μm, or 96% of cells with an accuracy of 5 μm. Using $^{3}He^{2+}$ ions (which are less easily scattered), 99% of cells are targeted with an accuracy of ±2 μm [12]. The spatial resolutions achieved by the collimated facilities at RARAF and at JAERI are ±3.5 and ±5 μm, respectively.

The statistical nature of most radiobiological assays means that it is often necessary to irradiate many thousands of cells to establish the underlying dose-effect with sufficient accuracy. It is essential therefore that the process of target identification, alignment and irradiation are both automated and rapid. Both the RARAF [4] and Gray Laboratory [2] facilities have advanced cell recognition and alignment capabilities. Typically, up to 4000 cells per hour can be located and irradiated using our facility, and a recent study involving cell transformation performed at RARAF required 260,000 cells to be individually identified and irradiated [13].

3. Studies related to low-dose radiation risk

The use of microbeams is now seen as one of the primary experimental strategies for investigating the cellular basis of hazards associated with low doses of charged-particles. For example, at dose levels that generally apply in environmental exposure to radon, virtually no cell receives more than one charged-particle traversal. By using a microbeam, the biological effect of *exactly* one α-particle can be investigated in an in vitro system. The oncogenic potential of a single α-particle has been measured by Miller et al. [13] using the RARAF microbeam. They irradiated C3H10T1/2 mouse fibroblast cells with either an *exact*, or an *average* number of α-particles and measured the transformation frequency (per surviving cell). When an exact number of particles are used, their results showed that the risk associated with exposure to a single particle is not significantly higher than that for zero dose, suggesting that extrapolating to low doses from multiple traversal data will significantly overestimate the risk of radon exposure at domestic levels, although caution is advised before applying this result to humans. By contrast, a study using a collimated microbeam at Pacific Northwest Laboratory, USA (currently being rebuilt following relocation to Texas A&M) measured chromosome damage (by scoring micronuclei induction) in CHOK1 cells following exposure to controlled numbers of 3.2 MeV α-particles and found that the amount of chromosomal damage per unit dose was similar to that resulting from exposures to α-particles from other types of sources [14]. They suggested however that additional studies are needed to ensure that each cell scored received the same number of nuclear traversals.

Another advantage of using a microbeam is that it is possible to assay radiation damage on a cell-by-cell basis, thereby avoiding the statistical uncertainty that arises from some conventional assays (such as clonogenic survival). In combination with precise particle delivery, the microbeam is therefore ideally suited to investigating the survival of cells at low doses. The Gray Laboratory facility has been used to measure the survival of V79 mammalian cells following exposure to 3.2 MeV protons at doses below 1 Gy (between 5 and 50 proton traversals per cell). At the lowest doses, the survival curve is very steep, indicating that the cells are very sensitive. Beyond about 10 protons per cell, the curve becomes less steep as the cells exhibit increased resistance to the radiation (the surviving fraction after 10 protons is 0.85, and after 50 protons is 0.72). This phenomenon, known as 'low-dose hypersensitivity' [15] has been shown previously for other radiations. It has been proposed that the onset of reduced radio-sensitivity may indicate that an inducible repair mechanism has been triggered.

4. Targeting sub-cellular regions

A major advantage of using microbeams is the ability to localise the radiation to regions of interest within the target cells or tissues. Many questions about radiation effects at the cellular level revolve around targets and pathways required for various biological responses. For example, triggering pathways for apoptosis may occur from external stimuli or direct damage to cellular DNA with different signalling pathways involved. For nuclear DNA, damage responses may be heterogeneous across the nucleus leading to differential damage expression.

Several studies, using microbeams have shown evidence for the cell cytoplasm being an important target for biological effects. Using the RARAF facility, Hei and colleagues [16] targeted the cytoplasm of human–hamster hybrid A_L cells with α-particles and monitored mutation expression. An increased production of mutations was observed after 4–16 particle traversals, and with a reduction in cell survival to around 80%. They also observed that the molecular spectra of these mutations were similar to spontaneous mutations that occur in un-irradiated cells. Further studies suggested that the induction of these mutations were dependent on the production of reactive oxygen species (ROS).

Studies at the Gray Laboratory using primary human fibroblasts have shown that targeting the cell cytoplasm with 5 helium-ions, leads to ROS production and chromosomal damage in the form of micronucleus induction [17]. These studies have been performed using dual staining with Hoechst and rhodamine 123. Evidence for chromosomal instability at delayed times after cytoplasmic irradiation is also observed although the relationship between this and ROS production is not clear.

5. Targeting individual cells within a population

In the past decade, several experiments have shown that interactions occur between irradiated and neighbouring non-irradiated cells. For example, Nagasawa and Little [18] showed that by delivering low doses of α-particles (from a conventional source) to a population of cells, such that less than 1% of the cells were traversed by a particle, then higher levels of DNA damage were produced in greater than 30% of the cell population. This form of non-targeted response has been termed the bystander effect and could lead to non-linearity in the dose-response of cells in the low-dose region, with implications for our understanding of the risk associated with low-dose exposure to ionising radiations.

There is a clear application for microbeams in the study of the bystander effect, and it should be noted that since only a few cells are targeted, this type of investigation could be undertaken without the requirement for automated cell finding and aligning systems that are essential for other types of microbeam-related work. Studies at the Gray Laboratory using primary human fibroblasts have shown that targeting a single cell within a population of 600–800 cells with a single helium-ion leads to an additional 80–120 damaged cells (scored as cells containing micronuclei) being produced uniformly across the population [19]. The level of damaged cells produced was found to be independent of the number of helium-ions targeted through the cell nucleus and to the number of cells targeted (up to 25% of the total number of initial cells). A similar approach using the RARAF microbeam has shown a radiation-induced bystander response for the production of mutations at the CD59 locus in the A_L cell line with a 30% higher mutation frequency than that assumed from the fraction of cells hit [20].

Our own studies have now been extended to tissue models to start to understand the role of cell-to-cell communication and tissue architecture on radiation response. Our preliminary work has been performed with sections of human or porcine ureter where a 4–5 cell layer of uroepithelium surrounds the lumen of the ureter. Experiments have been performed targeting individual protons or helium-ions into the epithelial layers of the tissue or into specific uroepithelial cells within explants. A significant bystander response is observed which, in contrast to the cellular studies, leads to several thousand additional damaged cells being produced.

6. Summary

Without doubt, interest within the radiobiological community in the potential of the single-cell irradiation technique has grown considerably over the past few years. Those microbeams currently in routine operation have been developed around established accelerator facilities, and in the first instance, have opted for collimation to achieve a micron-size particle beam. A new generation of single-cell irradiation facilities are now emerging, based on established analytical focusing microprobes. This brings with it a new set of problems, not least the scattering produced by the vacuum exit window, and the unfavourable horizontal configuration normally encountered with such facilities. An increasing amount of microbeam-related radiobiological data is now appearing in the literature. New findings have reported on the risk associated with exposure to environmental levels of radiation (including exposure to radon), and on the induction of non-localised damage through *inter*- and *intra*-cellular signalling. The contribution made by microbeams to radiobiology can only be expected to grow as new facilities begin operation.

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