An Overview of the Microbeam and Its Applications as Probe in Biological Systems

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Abstract: A micro beam is a very thin beam carrying usually protons, alpha particles or heavier particles with a size of ranging micrometers or smaller, corresponding to the cell size or the cell with a combination of techniques for locating living cells or sub-cell objectives (cell organelles), allowing the possibility of exposure to continuous radiation of these fast targets. This article basically reviews both technological aspects of modern single-cell micro beams and their applications. The recent concerns about micro beams started with the intrinsic problems in Radom, where the cells are affected by zero or an alpha particle. Micro beams allow the cells to be individually irradiated with precise particles. At the very early age of micro beams, environmental problems related to these radiations was impressed widely, namely by signaling as a result of cell injury. The focus was dedicated to two aspects of micro beams: first, to increase the sensitivity of detection of organelle targets such as the cytoplasm and mitochondria, and second, the exposure of some of the cells to micro beams specifically, allowing to check the conditions directly and identify messages regarding to biological response between irradiated and non-irradiated cells.
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1. Introduction

What happens in mammalian cells or adjacent cells when they are exposed to micro beams?

This fundamental question resulted in the development of single particle micro beam devices for single cells. In order to study the effect of ion radiation on a single cell, such as Bystander effect (Ponnaiya et al.; 2004), the machine had to be in a single cell scale, allowing to locate the organelle cell, and prescribe a precise dose in the target cell (Pehrson, 2002). The accuracy of a single cell single ion micro beam is defined as the delivery of at least a single ion in each cell nucleus, and in particular is applied in the study of the impact of Radom (Miller et al., 1999). Catalyst particles with ion light specificity or collimator can reproduce micro beams with smaller charges than the cell nucleus. In conjunction with imaging, positioning, and positioning techniques, a microbeam can be a powerful tool for controlled micro-beam irradiation test on cells. Although studies of cell exposure go back to 1950s (Zirkle and Bloom., 1995), but Columbia University (Pehrson, 2001) is the leader in the field of single-cell ionic group microbeams. Since then, there has been significant increase in the number of plans and operational microbeam devices. The optical ion microbeam accelerator device in radiological investigations (RARAF) at Columbia University is a perfect example for microbeam devices.

Microbeam systems in Radiobiological Research Accelerator Facility (RARAF)

RARAF microbeam platforms at Columbia University, are composed of a microbeam-based device to an array of micron-sized exposure probes. Microbeam platforms include: microbeams II (electron focusing system), neutron microbeams and micro spot UV (Xu et al. 2011). All of these systems have a common recipe including imaging, targeting, and irradiating. In summary:

1. The cells go under imaging process and the center gap is recorded by the control program.
2. The cells are accurately placed in irradiating position.
3. The cells are irradiated based on a predetermined dose, and
4. The next cell is brought to the site of exposure. (Bigelow et al., 2010).

Microbeam II

RARAF's principal microbeam system, Micro beams II is consisting of a quadrupole electrostatic lens, which is capable of producing submicron diameter beams in air (Bigelow et al., 2008). The electrostatic lens system consists of a quadrupole lens system, designed by Dymnikov (Dymnikov et al., 2000). Incentives for developing a microbeam systems with focused ion beams are:

a. Solving problems related to halo artifact with aperture-based microbeam systems

b. Improving the rate of sub-micron radiation beam on the target. While the structure of organelles is consistent with Sharp sub-micron radiation, the

sustainability of landscape designs in vertical microbeam II has been investigated.

To demonstrate the resolution of the microbeam II irradiator at RARAF, the letters "NIH" were irradiated onto a single live cell nucleus. The cells for this demonstration were HT1080 human Fibro Sarcoma cell nuclei containing GFP-tagged XRCC1 (singlestrand DNA repair protein). These cells were plated on microbeam dishes and kept under physiological conditions during the irradiation and imaging phases. A single cell nucleus was irradiated in an "NIH" dot-matrix stage-motion pattern with ~200 6-MeV alpha particles per spot. The pattern was reproduced via precision stage motions to irradiation locations. Typical spacing between points along each letter was 1 μm.

As DNA damage occurred in the cell nucleus, XRCC1 repair protein formed foci at the damage sites. Immediately after the irradiation, a Z-stack of 21 widefield fluorescent images was acquired using a waterdipping objective (60X 1.0NA), and a step size of $\Delta Z = 0.5$ μm, to reveal GFP concentrations in the nucleus exposed with the "NIH" pattern and in the neighboring (control) cell nuclei. Autoquant software was used to deblur the images in a post-processing step – result shown in Figure 1 (Bigelow et al., 2010).

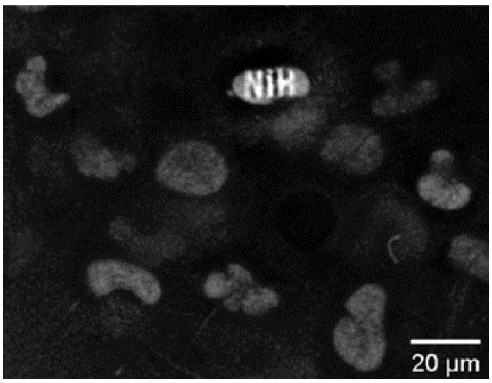

Figure 1. "NIH" written on a single cell nucleus with the RARAF microbeam.

Permanent Magnetic Microbeam (PMM)

The permanent magnetic microbeam (PMM) shown in figure 2 is a second focused charged-particle microbeam developed at RARAF (Garty et al., 2006). It uses permanent magnetic quadrupole lenses (STI Optronics, Inc., Bellevue, WA) whose strengths are tunable by varying the insertion of permanent magnets into the shaped yokes (Gottschalk et al., 2003). Originally, the project was carried out by use of a static ^{210}Po microbeam source. In the next step the system was used as a test-based accelerator (Harrison et al. 2007), which increased security reducing the exposure to associated plutonium ions. The use of ion source as accelerator, allows the use of heavy beams and reducing deviations in smaller spots. The application of PMM as the new microbeam source in RARAF, has led to economic justification of radiobiology experiments, because it excludes purchase of a particle accelerator and support electronics, and despite the electrostatic and electromagnetic lenses, PMM does not need high voltage suppliers and cooling systems.

Presently, the PMM is used for cell-irradiation experiments when development projects occupy the microbeam II endstation or beamline. Also, the PMM is used for development of point & shoot technology as well as microfluidics-based flow and shoot technology (FAST) (Bigelow et al., 2010).

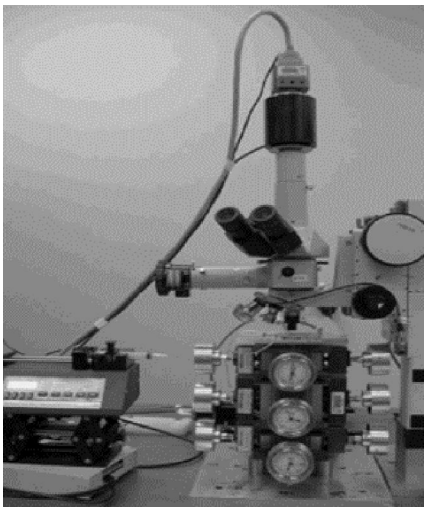

Figure 2. PMM and the Components.

X-ray Microbeams

Adding fine beams of X-ray for low linear energy transfer (LET) of cells at RARAF (Harken et al., 2008), has expanded the category of microbeam radiations from charged particles to electromagnetic radiation. The approach for the production of X-ray, is using particle-induced x-ray emission (PIXE). In a two-step process, proton beams generated by the accelerator, impinged a titanium target embedded in cooled copper rod to produce X-ray emission. Cells are positioned at the focal point of the X-ray using an irradiation endstation in the RARAF Model. In this two-step process in the production of a micro X-ray, the first stage involves the focusing of the proton beam and the second stage is to focus the X-ray (Figure 3). In the first stage, an electrostatic quadrupole quadruplet lens focuses protons from an object distance of 5.77 m to an image distance of 14 cm, where a 100 micron diameter proton spot impinges on a titanium target face cut at a 70-degree angle to the proton incidence. With this geometry, part of the 4.5 keV Kα titanium characteristic x-rays are emitted in the vertical direction toward the zone plate

and appear to originate from a 100 x 35 micron elliptical spot. In the second stage, the X-ray microbeams exit the vacuum system through a beryllium window into a helium-filled chamber and the focused x-ray beam profile is measured using a knife-edge scan similar to the one used to determine beam profiles in the charged particle microbeams (Bigelow et al., 2003). The main difference here is that we can control the effects of radiation.

Recently the size of the X-rays available is approximately 8 × 3 microns which is sufficient for the nucleus of a single cell, and for a radiation dose rate of 1mGy /S available, early biological experiments are expected to start.

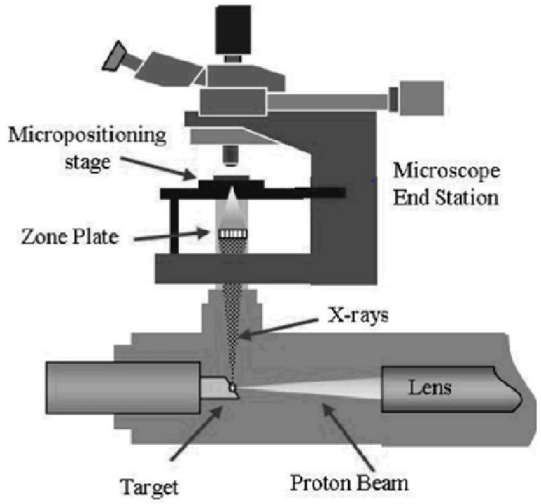

Figure 3. Schematic diagram of the x-ray microbeam.

Neutron microbeams

To complete the platforms for charged particles and electromagnetic microbeam irradiation at RARAF, we are developing neutron microbeams that can be delivered to the target cells. The rationale for developing the neutron microbeams is to investigate effect of comparable to those within commercial nuclear reactors that people may be exposed. In this design, neutrons from through nuclear reactions between beryllium and lithium and an electrostatic quadrupole quadruplet lens focuses the proton at the point of ten microns diameter on the lithium target where nuclear reaction occurs (Figure 4). The near-threshold reaction provides a relatively high neutron yield.

The neutron microbeams size is determined by imaging alpha particles on a lithium carbonate coated fluorescent nuclear track detector (FNTD) at the site of cellular irradiation. The alpha particles are generated through lithium reaction on the layer covered with FNTD. The sequence of the alpha particles on FNTDs can be captured by laser scanning microscope imaging techniques. The excitation wavelength is 335 or 620 nm and emission wavelength is 750 nm. To avoid the prolonged turnaround time required for imaging commercial FNTD, photomultiplier tubes are used to allow inhouse FNTD imaging with RARAF's multiphoton microscope (Bigelow et al., 2008).

Challenges in application of microbeams

Microbeams were originally designed for overcoming traverse cells problems in Radiobiology experiments. Clearly, if a dose of charged particles is irradiated to a group of cells, the number of traversed particles will not be identical, and therefore will vary from one cell to another. This problem is more serious especially for high-LET ions, such as a-particles and heavy ions, where at low doses the fraction of cells receiving no traversals at all can be very high. The effectiveness of single particle traversals is one of the main issues of radiation protection both on Earth and in space (Durante 2009). The possibility of targeting single cells with a predefined number of particles motivated the construction of the first microbeams (Gerardi 2009). This soon made possible the direct study of effects of single traversals through the cell nucleus or cytoplasm and evidence for the bystander effect (Zhou et al. 2000). However, several other applications were soon introduced and technologies rapidly improved. With the introduction of soft X-ray microbeams (Folkard et al. 2001), it became possible to compare low- and high-LET radiation and to improve the subcellular resolution. Some of these new topics and their current status are summarized below.

DNA damage

Use of microbeams to visualize recruitment of proteins involved in damage detection, signaling, and repair began early after the turn of the century (Tartier et al. 2003). Hauptner et al. (2004) introduced use of geometric irradiation patterns and demonstrated that the pattern of emerging protein foci reflects the irradiation pattern. Irradiation in geometric patterns also allowed the analysis of the effects of sequential ion micro irradiation and the detection of the competition effect, which highlights the turnover and binding characteristics of the different DNA repair molecules (Greubel et al. 2008, Fig 4).

Visualization of DNA damage in subnuclear targets was demonstrated initially at GSI (Heiss et al. 2006) and is today one of the main research topics. Moreover, the subnuclear targeting with heavy ions makes possible to study heterochromatic and euchromatic compartments directly (Jakob et al. 2011, Fig. 5).

The possibility of live cell imaging on the microbeam line will further enhance these possibilities (Hable et al., 2009). For example, the kinetics of recruitment of different proteins can be investigated in

individual cells and compared (Mosconi et al. 2011). The DNA damage/repair studies will certainly in future represent one of the main focuses of the microbeams with several different options. Given that the vast majority of data on kinetics and mutual dependence of protein recruitment to damage sites gathered so far has been obtained using laser micro irradiation (Bekker-Jensen and Mailand 2010), it will be particularly important to systematically investigate whether the data obtained after laser irradiation hold after ionizing irradiation.

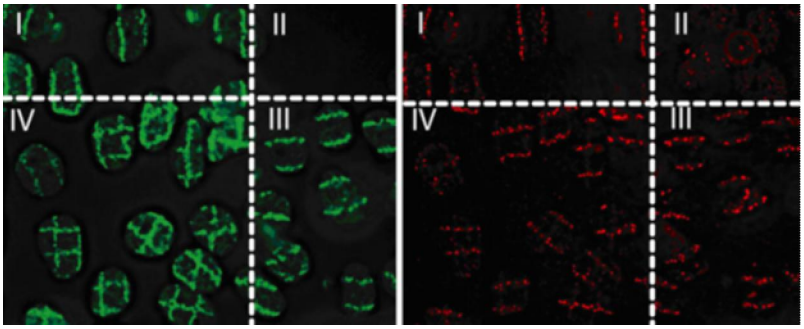

Figure 4. Competition effect revealed by sequential irradiation with single oxygen ions delivered in line-wise patterns at the Munich microbeam SNAKE. Hela cell monolayer was first irradiated in horizontal line pattern and after 45 min re-irradiated in vertical line pattern. Immunofluorescence detection of gamma-H2AX (green) reflects the irradiation pattern (first irradiation only in quadrant III, second irradiation only in quadrant I, both irradiations in quadrant IV, no irradiation in quadrant II). In contrast, in cells irradiated twice, 53BP1 foci (red) develop only at damage sites induced during the first irradiation, but not in response to the second irradiation (see quadrant IV), while they develop readily in cells that received only the second irradiation (see quadrant I). From (Greubel et al. 2008)

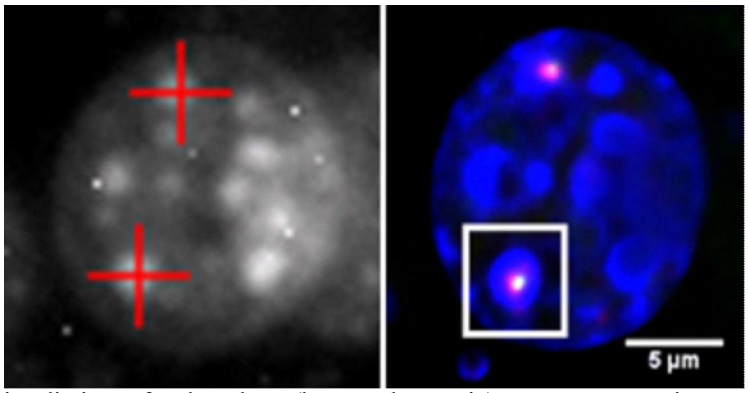

Figure 5. Microbeam irradiation of subnuclear (hetereochromatic) compartments in mouse embryo fibroblasts (MEF) with heavy ions at the GSI microbeam. The left image shows the aimed targeting of chromocenters (red crosses) for single ion irradiation using Hoechst 33342 (gray scale) as a marker in nuclei of living MEF cells. The right-hand image shows the same nucleus after fixation at 5 min postirradiation. DNA damage-induced foci of the repair factor XRCC1 (green) and gH2AX (red) are clearly visualized at the sites of ion traversal. Both proteins co-localize within each of the targeted chromocenters (blue: DAPI DNA staining). From (Jakob et al. 2011)

Tissue and animal models

The studies of bystander effect have dominated for many years the applications of microbeams (e.g. Zhou et al. 2000). However, experiments with cell monolayers are limited and do not take into account the complex tissue responses. Three-dimensional models can reproduce many of the tissue characteristics in vivo (Griffith and Swartz 2006) and are therefore ideal targets in microbeams for studying non-targeted effects. So far, mostly skin constructs have been used at microbeams (Schmid et al. 2010; Miller et al. 2011), but new organotypic slice culture methods offer the possibility of irradiating parts from all kinds of human tissues and to study their late response (Merz et al. 2010). The tissue models at microbeams can be very useful to clarify the role of cell signaling and tissue remodeling in radiation response.

Although traditionally hampered by the limited range of particles and photons used at microbeams,

animal models are now also used. So far, these studies focused on very small animals, such as silkworms and nematodes (Bertucci et al. 2009), but can provide important insights on long-range non-targeted effects, beyond the possibility of 3D tissue targets.

Generation of ultra-high dose rates

A rather recent application of microbeams is to produce very short pulses (a few Gy in ~1 ns), similar to the conditions expected to occur in particle irradiation setups with laser-driven accelerators (Dollinger et al. 2009). Studies on radiobiological effects of ultra-high dose rates are a prerequisite for potential future applications of laser driven particle acceleration in radiotherapy. Recently, it was shown that irradiation of mouse tumor models at microbeams can be useful in preparing this new therapy modality (Greubel et al. 2011).

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