Development of an Electron Microbeam for Cell Culture Studies

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INTRODUCTION

Current radiation protection standards have traditionally been based upon a linear no threshold extrapolation from high doses where real risk can be experimentally or epidemiologically observed. However, it is argued that this model can overestimate the risk at the low doses that are received at research or industrial facilities. Some have also argued the opposite, that the risk is actually underestimated. In either case it would be helpful to better understand the true level of risk at such doses; either research costs could be significantly reduced, or workers' health could be more accurately ensured.

Unfortunately, there is no direct method to measure this risk to humans since the natural background rate of cancer, the primary effect of radiation exposure, is much higher than the added risk we are willing to accept from occupational and other man-made exposures. We must therefore use animal and cell culture research in order to establish any alternative to the linear extrapolation currently in use. Due to complex interactions between cells and tissues within an organism as well as variations in regulation of proliferation, efficiency of repair and other radiation responses, a very complete description of the processes involved in the radiation response is needed in order to achieve a more accurate estimation of risk at low doses. Microbeam cell irradiation studies can directly address this by allowing correlation of a particular response to a particular direct dose interacting in the actual irradiated cell or a neighboring cell.

Recent biological studies have produced convincing evidence that cells which have not been directly hit by ionizing radiation often undergo changes such as an increased rate of apoptosis, increased levels of repair-related proteins, and increased malignant transformation in response to radiation damage in neighboring cells.[1,2] It is not evident whether this "bystander effect" is present in intact tissues or not. If it occurs in tissues and animals, it may either increase or decrease the risk of cancer.

While about half of the dose equivalent received by the population is produced by high-linear energy transfer (LET) radiations, approximately 90% of the absorbed dose is delivered by gamma rays and other low-LET radiations. Therefore, it is at least as important to gain an understanding of the biological consequences of low-LET irradiations as high-LET irradiations. Although neighboring cell effects have been observed almost exclusively as a result of high-LET irradiations, this may be because the experimental conditions needed to see bystander effects with low-LET radiations produce such a low dose that the signal, if present, would be nearly impossible to separate from the stochastic variation in biological response. Thus targeted low-LET irradiation techniques, using a microbeam apparatus, become necessary to determine the number of electrons and other low-LET radiations which can trigger radiation-induced effects in cells which have not been directly irradiated.

DESCRIPTION OF THE ACTUAL WORK

To study the microdosimetry and bystander effects of low-LET irradiations, we have recently constructed an electron microbeam apparatus. While the interaction of electrons with tissue necessarily produces a large degree of scattering, the experiments needed to evaluate the efficacy of bystanding cell effects on low LET irradiation do not require targeting a specific portion of a cell or limiting the exposure to a single electron. Currently, we utilize a narrow line of electron tracks imposed upon a cluster of cells. Readings of the biological response are taken from the cells that were distant from the irradiated line at the time of exposure.

Electron Microbeam Design

A simple electron gun consisting of a regulated 100kV power supply, a three-section insulating tube and a low power tungsten filament provides the electron beam. An internal ring-shaped Faraday cup gives a relative measure of the beam current in real time. Beam currents of approximately only 0.1 na/cm^2 are needed to

produce 100 particles per second through the 5 μm diameter collimator.

A variation of the glass capillary method used at the Gray laboratory on positive ion beams is being used in our apparatus. The collimator is mounted in a small tube, the terminus of which is positioned at the center of a segment of a sphere which rests on three bearings, allowing the angle of the collimator to be adjusted without moving its exit point in the plane where the cells will be irradiated. Collimators ranging from 5 to more than 300 μm can be used.

Targeting and motion control are provided via computer control of a Newport driver (model ESP300) governing two independent actuator-powered X- and Y-directional stages. Special mylar-bottomed cell-culture dishes are used to allow irradiation from below and to ensure positional reproducibility. The cell cultures are observed during the experiments via a dedicated video microscope, allowing for accurate positioning of the cell clusters for line irradiation.

RESULTS

At this point, the electron microbeam has produced measurable beams up to 90kV, and beam has been directed through the collimator onto target cells. Some preliminary cell studies have even been performed using the apparatus. Currently, we are working on refining the accelerator tube design for greater beam stability at the low currents required for the biological studies.

REFERENCES

1. W. F. Morgan, *Radiat. Res.*, **159**, 567 (2003).
2. W. F. Morgan, *Radiat. Res.*, **159**, 581 (2003).