Radiation Protection Dosimetry
Vol. 99, Nos 1–4, pp. 283–286 (2002)
Nuclear Technology Publishing

MICROBEAMS: A POTENT MIX OF PHYSICS AND BIOLOGY. SUMMARY OF THE 5TH INTERNATIONAL WORKSHOP ON MICROBEAM PROBES OF CELLULAR RADIATION RESPONSE

D. J. Brenner and E. J. Hall
Center for Radiological Research, Columbia University
630 West 168th Street, New York, NY 10032, USA

INVITED PAPER

Abstract — Single-cell microbeam irradiators are of increasing interest to the biological community. The 5th International Workshop on Microbeam Probes of Cellular Radiation Response, which took place in Stresa, Italy, in May 2001, was attended by about 120 registrants, roughly evenly divided between physicists and biologists. Many new microbeam devices are now under development, and there has been a significant diversification of the biological questions addressed. Most current uses of microbeams have been to address radiobiological questions, but the advent of sub-micrometre targeting capabilities, and the development of new single-cell assays, point to the potential for microbeams to make an important contribution to biological, and not just radiobiological, studies.

INTRODUCTION

There is little doubt that the use of single-cell microbeams is of increasing interest to the biological community. The 5th International Workshop on Microbeam Probes of Cellular Radiation Response (see Table 1), which took place in Stresa, Italy, in May 2001, was attended by about 120 registrants, roughly evenly divided between physicists and biologists. Extended Abstracts are in the press in *Radiation Research*. What are microbeams? What can they do?

Essentially, a microbeam is a very narrow beam of radiation, of micrometre or submicrometre size, corresponding to cellular or sub-cellular dimensions. Together with appropriate integrated location techniques for individual cellular or sub-cellular targets, they allow rapid sequential irradiation of these targets, one by one, in individual cells. Charged particle microbeams also allow irradiation with exact numbers of particles, including one, circumventing the problems with conventional irradiations at low doses, where targets are traversed by a Poisson-distributed number of particles.

Why are we interested in microbeams? The recent explosion of interest in microbeams over the past decade was, in large part, driven by the interest in the radon problem. Whilst it was clear from studies of uranium miners that high doses of radon do cause lung cancer, it was less clear that such results could be directly extrapolated down to the domestic radon exposure situation, where target cells would be exposed either to zero or one alpha particle, but almost never to more than one. Microbeams allow cells to be individually irradiated with exactly one alpha particle (or exactly two, or more) — in this context one particle being the ultimate low dose.

However, as microbeams were built, refined, and used over the past decade, the biological questions that were addressed with them have broadened considerably. Two areas in particular have attracted much interest. One is the use of microbeams to address the sensitivity of sub-cellular targets, such as the cytoplasm. The other reflects the ability of the microbeam to irradiate some cells, but not others, in a group of cells and, if necessary, to keep track of which were irradiated and which were not. This allows a direct investigation of the so called bystander effect, where signals from irradiated cells can apparently cause biological responses in neighbouring unirradiated cells.

To briefly summarise the Workshop, and to compare it with those that went before (see Table 1), one might conclude:

(1) Many new microbeam devices are now under development.
(2) There has been a significant diversification of the biological questions addressed.

We will briefly discuss both of these developments.

Table 1. Previous workshops on microbeam probes of cellular radiation response.

1. Gray Laboratory, London	1993
2. Pacific Northwest Labs, Washington	1995
3. Columbia University, New York	1997
4. Dublin, Ireland	1999
5. Stresa, Italy	2001
6. ?? England ??	2003

Contact author E-mail: djb3@columbia.edu

NEW MICROBEAM SYSTEMS

Three aspects really stood out at the Workshop:

(1) The large number of new microbeam systems, which are under development. These are summarised in Table 2. Perhaps the most noticeable aspect is that most of these systems have not as yet incorporated the necessary biological target imaging and placement hardware and software. Our own experience at the Columbia University microbeam facility is that the integration of the physics and biology is both the most challenging aspect of microbeam development, and also the most time consuming. Really the job can only be done through a genuinely multi-disciplinary effort in which physicists and biologists need to communicate and interact very well.

(2) The development of electron and photon microbeams. Much of the early interest in microbeams, in large part because of the radon issue, was for high-LET radiation. More recently there has been interest in probing the LET response of phenomena such as the bystander effect. At the Workshop, data from proton, soft X ray, and electron microbeams were all discussed. Each system has advantages and disadvantages. Electron beams of course, are subject to considerable scatter, though Monte Carlo simulations presented at the Workshop suggest that individual cells can be irradiated with a relatively low probability of scattered electrons hitting an adjacent cell. Ultrasoft X rays, because they interact through photoelectric effects, have no scattering problems, and can be focused extremely well, though interpretation of results is complicated because of the strong attenuation in energy deposition from the front to the back of a cell; these soft X rays are of intermediate LET (20 and 40 $keV.\mu m^{-1}$ for aluminium and carbon K X rays, respectively). Protons do not suffer from scattering or attenuation problems, but need to be of fairly high energy to produce a low-LET beam. For example, a 4 MeV proton beam is required to produce an LET of 9.5 $keV.\mu m^{-1}$ (ICRP 'defines' low LET as below 10 $keV.\mu m^{-1}$).

(3) A general move from collimated charged-particle systems, where the microbeam is essentially defined by an aperture, to focused systems, either electrostatic or magnetic. There is a good reason for this move. Collimated systems have a lower limit, around 2 μm, as to the possible beam size; this is related to the amount of scattering produced by the inside walls of the collimator itself. This scattering produces a penumbra to the beam profile, which becomes more and more important as the collimator diameter is decreased. By contrast, focused beams do not suffer from these scatter problems and produce, at least in principle, a sharp beam without a penumbra, allowing the possibility of sub-micrometre microbeams.

BIOLOGY WITH MICROBEAMS

There was a considerable focus at the Workshop on bystander effects, which is perhaps not surprising as (a) this phenomenon has the potential to challenge many preconceived notions regarding radiation damage mechanisms and thus risk estimation, and (b) as discussed above, microbeams are, in many ways, ideal tools for probing bystander effects. The most obvious situation where bystander effects might be important relates to domestic radon risks, where a target cell traversed by

Table 2. Microbeam systems reported at this Workshop*.

Laboratory	LET	On line?	Biology?
Gray Laboratory, London, UK	low, high	Yes	Yes
Gray Laboratory, London, UK	soft X	Yes	Yes
Columbia University I, New York, USA	high	Yes	Yes
Columbia University II, New York, USA	low to very high	No	No
JAERI, Takasaki, Japan	high	Yes	Yes
PNL, Richland, Washington, USA	low	Yes	No
PTB, Braunschweig, Germany	low, high	Yes	No
CENBG Bordeaux Gradignan, France	low, high	Yes	No
INFN-Lab. Naz. Legnaro — Padova, Italy	low, high	Yes	No
Universität Leipzig, Leipzig, Germany	low, high	Yes	No
MIT, Boston, USA	low, high	Yes	No
Technische Universität München, Garching, Germany	low, high	Yes	No
INFN — Sez. di L'Aquila, L'Aquila, Italy	soft X	No	No
INFN — Sez. di Padova, Padova, Italy	high	No	No
LBL, Berkeley, USA	very high	No	No

* At least one other system (at Texas A&M University) is operational but not reported at this Workshop.

an alpha particle is very likely to be surrounded by similar cells, which have not. Could the untraversed cells show deleterious effects caused by signals from the directly damaged cell?

There are many other situations where bystander effects might be important, such as individuals exposed to low doses of neutrons (for example from photoneutrons in high-energy photon radiotherapy, or cosmic ray induced neutrons in aircraft). If low-LET radiations can cause significant bystander effects, the implications could be significantly broader.

In fact bystander effects were first identified before the advent of the current generation of microbeams, in low-dose studies with broad beams of alpha particles. Further studies followed in which the medium surrounding irradiated cells was transferred to unirradiated cells. Because of its specificity, however, the microbeam has proved a potent tool for quantitative investigations of bystander studies. For example, results were reported from the Gray Laboratory in which a single cell at the centre of many cells in a Petri dish was irradiated, and increased responses observed in many cells throughout the dish, relatively uniformly over the whole dish. In another example, in experiments reported from Columbia University, a small fraction of the cells on a dish were irradiated, and all the cells then followed and assessed *in situ*, maintaining the information for each cell as to whether it was or was not irradiated.

In summary, the evidence that bystander signals can cause a variety of biological effects seems unequivocal. There were some suggestions at the Workshop that the bystander effect might have an evolutionary origin as a protective cell-killing mechanism, but this seems unlikely, given the range of deleterious non-lethal endpoints that bystander signals can apparently induce. What does seem clear is that:

(i) An irradiated cell can indeed send out a signal that can lead to a response in a bystander cell.
(ii) The bystander phenomenon seems to be a binary effect — more signal generally does not result in an increased response.
(iii) Not all irradiated cells can respond to bystander signals.
(iv) There are probably several different mechanisms underlying bystander effects. In some instances, particularly in experiments on confluent or near-confluent cells, gap-junction communication is clearly important. In other studies with more sparsely plated cells, the bystander information appears to be transmitted through a vector released into the surrounding medium.

Early results using proton- and ultrasoft X ray microbeams were also reported, probing low and intermediate LET ranges, respectively. The evidence so far is that some of the bystander effects seen at high LET are also induced by low-LET radiation. Whether a single low-LET proton can produce a bystander signal is yet to be definitively established. Results from the Gray Laboratory suggested that bystander-induced cell killing was not seen after irradiation with single protons, though the observed absence of this effect may, of course, only reflect the necessarily limited sensitivity of the biological assay.

Another issue that was elegantly addressed with microbeams was whether cells, which were previously exposed to low doses of low LET radiation (as are all the cells in our body), could still respond to bystander signals. It now seems clear that lightly irradiated cells can still respond to a bystander signal, though in some cases an 'induced repair' effect was seen which produced the opposite effect (i.e. a decrease in effect) to the bystander response.

In terms of endpoints, most of the common endpoints used in radiobiology can be assayed after microbeam irradiation — though if *in situ* tracking is required of which cells have been irradiated and which have not, only a limited number of endpoints are currently possible. At the Workshop, microbeam results were reported for chromosomal damage, mutation induction, cell killing, *in vitro* oncogenic transformation, p-21 focus formation, and DNA fragment size, as well as some *ex vivo* assays in a three-dimensional tissue.

WHERE NOW?

In terms of microbeam development, it is clear that the focus needs to be more at the 'business end' of the machine, i.e. the software, hardware, and biology associated with automated imaging and locating of the desired cellular or sub-cellular targets, and sequential movement of these targets into the beam. Development in these areas may well be falling behind our capabilities to simply produce narrow radiation beams *in vacuo*.

In terms of biological developments, microbeams currently offer a powerful tool for probing the mechanisms of the bystander effect. An important biological advance here will be the use of '*ex vivo*' tissue targets, where the cells maintain (at least at some level) the same cell-to-cell communications as in the '*in vivo*' situation. A model system reported by Belyakov and colleagues involving 3-D microbeam targeting of porcine tissue fragments will likely stimulate the development of other such systems.

In the near future, as microbeams do move into the sub-micrometre realm, there will surely be an increased emphasis on biological endpoints that can be scored in single cells. Some were reported at the Workshop, such as the application of single-cell PCR, but many more will be needed to fully take advantage of the exquisite targeting abilities of the latest microbeams. In this regard, the information from a recent Workshop held in Bethesda (*Probing Individ-*

ual Cells: Applications to Signaling, Structure and Function, March 2001; Proceedings in *Radiation Research* **153**(2), 220–238 (2002) is highly pertinent here, and again points to the potential for microbeams to make an important contribution to biological, and not just radiobiological, studies.

ACKNOWLEDGEMENTS

This work was supported by grants RR-11623, CA-49062, and CA-37967 from the National Institutes of Health, and by grants DE-FG02-98ER62686 and DE-FG03-00-ER62909 from the US Department of Energy.