Microdosimetry of Electron Microbeams

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ABSTRACT

Track structures of 25, 50, and 80 keV primary electrons, simulated by the detailed-history Monte Carlo method, were analyzed for the frequency distributions of energy deposited in spheres with a diameter of 1 μm placed in a cylindrically symmetric array around the projected initial direction of the primary electron. The frequency mean of specific energy, the dose mean of lineal energy, as well as the median and variance of log normal functions fit to the dose distributions were calculated as a function of beam penetration and radial distance from the projected beam axis. Given these data, the stochastics of dose and radiation quality for micrometer-scale sites targeted by a medium-energy electron microbeam can be predicted as a function of the site's location relative to the beam entry point.

Introduction

Radiation-induced bystander effects (RIBE), responses of cells not traversed by particles of a radiation field, have challenged the conventional wisdom that health risks are determined by the responses of hit cells to damage in their DNA (1). An understanding is emerging that places RIBE in the broader context of the interactions of cells with their microenvironment (2) that generate a coordinated multicellular response in irradiated tissue with the objective of modulating cellular repair and death programs. To fill in the details of this picture, experimental tools are needed that unambiguously distinguish hit cells from close neighbors that did not absorb energy from either primary or secondary particles. Microbeam technology (3-7) is being developed to meet this need.

The large body of experimental data on RIBE has been the subject of several recent reviews (8-12). Most of these data were obtained in experiments with ionizing particles characterized by high linear-energy-transfer (LET). Most of the cells in a population exposed to a low fluence of high LET particles are bystanders. This property of high LET radiation was the basis for early work on RIBE (13-16) in which responses were observed in more cells than could have been traversed by high-LET particles. More recent studies with ion microbeams support the conclusions of the earlier work and allow more mechanistic studies through targeting of individual cells and subcellular components.

Data on bystander effects induced by low-LET radiation (reviewed in 17) have been more difficult to obtain. Early work by Mothersill and Seymore (18) showed that medium from γ-irradiated epithelial cells reduced the cloning frequency of unirradiated cells. Subsequent work, where individual cells plated at low density were targeted for exposure to α particles (19) or soft x-rays (20), led to the conclusion that soluble factors released by hit cells and also present in conditioned medium transmit bystander effects in the absence of cell-to-cell contact.

Microbeam studies (17,19, 20) have revealed several interesting features of bystander effects induced by media-transferable factors. First, within the range observable on a 5x5 mm^2 dish (about 3 mm), the probability that a bystander responded to soluble factors released by a targeted cell was independent of the distance between the cells; however, cells that responded to bystander signals by failing to form healthy clones were clustered relative to each other (20). These observations suggest that bystanders also release signals that triggers a chain reaction throughout the cell population.

Second, the number of bystanders that respond does not increase significantly with the number of hit cells. Targeting just one cell is sufficient to transmit a bystander signal throughout a 25 mm^2 dish and hitting more cells does not increase the strength of the that signal. Up to 0.2 cGy, the dose response for clonogenic survival of V79 cells was the same when one cell was exposed to carbon K-shell (C_K) x-rays as when all cells on the dish where targeted (20). Between 0.2 cGy and 2 Gy, survival after targeting one cell remained essentially constant at about 90%, while the dose response when all cells were hit with the same number of soft x-rays continued to decrease.

Third, for the endpoint of micronuclei formation, the bystander effect is strongly dependent on radiation quality. A single α particle traversing a single fibroblast (delivering a dose of about 0.1 cGy to the nucleus) stimulates the bystander response completely. The fraction of cells exhibiting micronuclei did not increase when up to 15 α particles were targeted to a single cell (19). C_K soft x-rays are considerably less effective at inducing this bystander response. For doses to the nucleus in the cGy range, α particles were 2-3 times more effective at inducing micronuclei in bystanders than C_K soft x-rays (20). Braby and Ford (21) have shown that cell lines which exhibit RIBE under α-particle and ultrasoft x-ray exposures but do not show these effects when exposed to a microbeam of moderate energy electrons, which also indicate a dependence on radiation quality. These findings suggest that under common exposure scenarios, where most cellular damage is delivered by low-energy secondary electrons, generation of a bystander signal is a stochastic endpoint that depends on the amount and quality of energy transferred to the hit cell.

Monte Carlo track-structure simulation (reviewed in 22, 23) has emerged as a general tool for investigating spatial patterns of energy deposition by charged particles. Application of the PITS code set (24) to electron microbeams with energies in the 25 to 80 keV range (25) suggested that selected cells could be irradiated under conditions where event-size distributions approached those of conventional low-LET radiation exposures (26). Wilson and coworkers (27) used the same set of track-simulation codes to characterize energy deposition by a 25 keV microbeam in terms of the probability of an event in a 1μm diameter sphere and the mean size of such events.

Recently, the PITS code set has been upgraded (28) to include condensed-phase effects in electron-impact inelastic-scattering cross sections (29). In this paper, we use the upgraded code to reexamine the microdosimetry of electron microbeams. Our computational approach is briefly presented in the Methods section. In the next section, we present and discuss our calculations of the probability to impart various amounts of energy to micrometer-size targets under microbeam irradiation by single electrons in the 25 to 80 keV energy range. We summarize our finding and discuss future work in the final section.

Methods

The recently updated PITS code set (28) was used to generate electron tracks at primary energies of 25, 50, and 80 keV. PITS produces a detailed-history of the simulated slowing down of primary electrons that includes all generations of secondary electrons. The path of an electron was followed until its energy dropped below a user-defined threshold of 10 eV. The spatial coordinates of all inelastic collisions were passed to a scoring algorithm that sampled the distribution of energy-loss events in spheres with a diameter of 1 μm placed in a cylindrically symmetric array around the projected initial direction of the primary electron. To maximize the use of each simulated track, equivalent sites (non-overlapping spheres at the same penetration and distance from the beam axis) were scored individually and results subsequently combined.

Frequency distributions in energy imparted, ε, were scored as a function of both forward (h) and lateral (r) penetration by analysis of at least one million tracks at each primary energy. The probability density $f(\varepsilon; r, h)$, where $f(\varepsilon; r, h)d\varepsilon$ is the probability per incident electron of depositing energy between ε and $\varepsilon+d\varepsilon$ in a sphere with center at r and h, was represented by the sum of two terms

$$f(\varepsilon; r, h) = f_m(r, h)\delta(\varepsilon) + f_1(\varepsilon; r, h). \qquad (1)$$

Integration of the first term, which includes the Dirac delta function, over the full range of ε gives the fraction of tracks, $f_m(r, h)$, that produce no energy deposition in the sphere at (r, h). When r and h are small, f_m is zero or small and becomes very close to unity at large r and h. The second term, $f_1(\varepsilon; r, h)$, is the density distribution of energy imparted conditional on some energy being deposited in the site; hence this term is defined for $\varepsilon > 0$ only. To calculate $f_1(\varepsilon; r, h)$, the total energy deposited in each virtual sphere of the cylindrical array was scored for each primary electron injected into a homogeneous water medium along the symmetry axis of the array.

Since it allows for tracks that miss the target, the probability density $f(\varepsilon; r, h)$ can be normalized to unity by an integral over ε that includes zero. The zeroth moment of $f_1(\varepsilon; r, h)$, excluding $\varepsilon = 0$, is the probability per primary electron that some energy is deposited in the site. This quantity, which we call the event frequency, is one or close to unity for sites at or near the beam entry point. Due to absorption and the geometric effect of increasing absorber volume, the event frequency becomes very small in sites far from the beam entry point.

The first moment of $f(\varepsilon; r, h)$ is the frequency mean of energy imparted, or specific energy if we introduce the random variable $z = \varepsilon/m$, where m is the mass of water in our 1 μm spherical sites. The first term on the right-hand side of Eq. (1) does not contribute to this moment, which can be expressed as

$$\bar{z}_F(r,h) = \int_0^\infty g(z;r,h)dz \tag{2}$$

where $g(z; r, h) = zf_1(z; r, h)$ is the dose distribution in specific energy. Histograms of specific energy scored in a cylindrical array of 1 μm spheres were fit by lognormal functions, defined here as

$$g(z;r,h) = \frac{\bar{z}_F(r,h)}{\sqrt{2\pi}\sigma(r,h)z} \exp\{-[\ln(z/\mu(r,h))]^2/2\sigma(r,h)^2\}. \tag{3}$$

Lognormal functions have been used previously (30) to give good two-parameter representations of charged-particle straggling distributions. Eq.(3) differs from the standard definition of lognormal functions (31), where ln(μ) rather than μ is used as a parameter. When μ is used as a parameter, all quantities in Eq.(3) that have units occur in dimensionless ratios. This facilitates changing the independent variable from energy imparted to specific energy and lineal energy.

Results and Discussion

Figure (1) shows the event frequency as a function of *r* and *h* calculated for a beam energy of 25 keV. The shape of this surface is similar to that reported earlier (27), which was calculated with a version of the PITS code set based entirely on gas-phase cross-section data (22, 24). Careful comparison of Figure (1) with our earlier result shows that the event frequency decreases more rapidly with increasing *r* and *h* when electron tracks are simulated with the new version of PITS that includes condensed phase effects in ionization cross sections and better treatment of elastic scattering.

Figure (2) shows fits of Eq.(3) to dose distributions scored for 25 keV primary electrons in 1 μm diameter spheres centered at representative values of *r* and *h*. For values of *r* and *h* larger than about 10μm, the shape of dose distributions is poorly defined due to the very low event frequencies. Nevertheless, absorption in the region of (*r, h*) where lognormal functions give a good representation of dose distributions accounts for more than 97% of the beam energy at 25 keV. Similar results were obtained at higher beam energies.

Figure (3) shows the frequency mean of specific energy $\bar{z}_F(r,h)$ obtained from fitting Eq.(3) to dose distributions in 1 μm diameter spherical sites centered at *r* and *h*. One can easily show that $\bar{z}_F(r,h)$ is proportional to the product of the event frequency and the average energy deposited in a site conditioned on some energy being deposited in the sphere (27,28). The latter is a slowly varying function of *r* and *h*; hence, the shape of the $\bar{z}_F(r,h)$ surface in Figure (3) at 25 keV is similar to the event frequency shown in Figure (1).

Comparison of results at different beam energies is facilitated by introducing scaled spatial coordinates $\rho = r$/p90 and $\eta = h$/p90, where p90 is the radius of a hemisphere that accounts for absorption of 90% of the beam energy. The p90 values calculated for 25, 50 and 80 keV were 8.65 μm, 27.1 μm and 56.2 μm, respectively. The most significant changes in $\bar{z}_F(\rho,\eta)$ with increasing beam energy are (1) greater statistical fluctuations at large ρ and η, and (2) a sharper peak at small values ρ and η. The former is due to greater fluctuation in the amount of energy deposited in a 1 μm diameter sphere by more energetic electrons. The latter is due to increased likelihood of forward scattering at higher beam energies.

Optimum values of fitting parameters μ and σ are shown in Figures (4) and (5), respectively, as functions of ρ and η. These parameters are not as strongly dependent on the position of the target sphere as $\bar{z}_F(\rho,\eta)$. Statistical fluctuations in the optimum values of μ and σ increased with beam energy, just as it did for $\bar{z}_F(\rho,\eta)$, but the only systematic change in μ and σ with increasing beam energy is that $\mu(\rho,\eta)$, the median of the distribution, decreases. A similar effect was found when lognormal functions were used to characterize the distribution of energy imparted to spherical sites by 0.3 to 20 MeV protons (30).

Lineal energy is an alternative description of energy deposition that may be useful in quantitative assessment of the responses induced in complex targets by low-LET microbeams because it more closely reflects the quality of the radiation. Lineal energy is defined (32) by y = $\frac{\varepsilon}{l}$, were ε is the energy deposition in single events in a volume with mean cord length l, which is 0.67 μm for the 1 μm diameter spherical sites used in our study. The dose distribution in terms of lineal energy, obtained by changing the independent variable in Eq.(3), is

$$g(y;r,h) = \frac{\bar{y}_F(r,h)}{\sqrt{2\pi}\sigma(r,h)y} \exp\{-[\ln(y/\mu'(r,h))]^2/2\sigma(r,h)^2\} \qquad (4)$$

where $\bar{y}_F(r,h)$ is the frequency mean of lineal energy and $\mu'(r,h) = \frac{m}{l}\mu(r,h)$

Let D(y) be the fraction of absorbed energy delivered with lineal energy less than or equal to y, then

$$D(y) = \frac{\int_0^y g(y; r, h)dy}{\int_0^\infty g(y; r, h)dy} = \frac{\int_0^y g(y; r, h)dy}{\overline{y}_F(r, h)} \qquad (5)$$

The dose probability density in lineal energy, $p_D(y)$, is the derivative of D(y) with respect to y; hence, $p_D(y) = g(y; r, h)/\ \overline{y}_F(r, h)$. The dose mean of lineal energy,

$$\overline{y}_D(r, h) = \int_0^\infty y\, p_D(y)\, dy \qquad (6)$$

is a non-stochastic quantity that characterizes the quality of the radiation interacting with the site. Figures (6) shows the variation of $\overline{y}_D(r, h)$ as a function of $\rho = r$/p90 and $\eta = h$/p90 for beam energies of 25, 50 and 80 keV. The shape of these surfaces and their dependence on beam energy are very similar to that shown in Figure 4 for $\mu(\rho,\eta)$. This should be expected since $p_D(y)$ is lognormal and the mean of a lognormal distribution (31) is related to its median and variance by

$$\overline{y}_D(r, h) = \mu'(r,h)\exp(\sigma^2(r, h)/2) = \frac{m}{l}\,\mu(r,h)\exp(\sigma^2(r, h)/2) \qquad (7)$$

From Figure (5), one sees that $\exp(\sigma^2(r, h)/2)$ is of order unity and not strongly dependent on *r* and *h* except near the origin.

Conclusions

Experiments with low-LET microbeam probes (17, 21) suggest that the amount of energy and quality of radiation absorbed by hit cells affect the responses observed in neighboring bystander cells. We have used Monte Carlo track-structure simulations to calculate the spatial dependence of microdosimetric quantities for an electron microbeam operated between 25 and 80 keV. The parameters of lognormal functions (31) fit to dose distributions for 1 μm diameter spherical sites at various lateral, *r*, and forward, *h*, penetrations are not strongly dependent on beam energy when *r* and *h* are scaled by the distance within which 90% of the beam energy is absorbed.

This study suggests that simple functions of beam energy and scaled forward and lateral penetration can be found that will allow the results of extensive track-structure simulations to be characterized in terms of $\overline{z}_F(r, h)$, $\overline{y}_D(r, h)$, and $\sigma(r, h)$. Given these functions, the probability of depositing a specified dose in a micrometer-scale target by a medium-energy electron microbeam can be predicted as a function of the targets location relative to the beam entry point. Since the energies deposited in a target by different primary electrons are statistically independent, dose distributions in spatially resolved targets for multiple-electron pulses can be obtained from the distributions for single electron tracks by standard convolution techniques. Work is in progress toward the development of such a global model for low LET microbeam dose distributions.

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Figure Captions:

Figure 1. Probability that some energy is deposited by a 25 keV primary electron in 1 μm diameter spherical sites as a function of beam penetration and radial distance from the beam axis.

Figure 2. Examples of lognormal functions (curves) fit to frequency distributions (histograms) of specific energy deposited in 1 μm diameter spherical sites located at various lateral (r) and forward (h) penetrations from the point of injection of 25 keV primary electrons.

Figure 3. Frequency mean of specific energy deposited in 1 μm diameter spherical sites by 25, 50, and 80 keV primary electrons as a function of lateral (r) and forward (h) penetration scaled by the distance from the beam entry point within which 90% of the energy is absorbed.

Figure 4. Median of lognormal fits to frequency distributions of specific energy deposited in 1 μm diameter spherical sites by 25, 50, and 80 keV primary electrons as a function of lateral (r) and forward (h) penetration scaled by the distance from the beam entry point within which 90% of the energy is absorbed.

Figure 5. Variance of lognormal fits to frequency distributions of specific energy deposited in 1 μm diameter spherical sites by 25, 50, and 80 keV primary electrons as a function of lateral (r) and forward (h) penetration scaled by the distance from the beam entry point within which 90% of the energy is absorbed.

Figure 6. Dose mean of lineal energy deposited in 1 μm diameter spherical sites by 25, 50, and 80 keV primary electrons as a function of lateral (r) and forward (h) penetration scaled by the distance from the beam entry point within which 90% of the energy is absorbed.