Radiation Protection Dosimetry Advance Access published April 16, 2015

Radiation Protection Dosimetry (2015), pp. 1–4 doi:10.1093/rpd/ncv146

MODELLING PROTON BUNCHES FOCUSSED TO SUBMICROMETRE SCALES: LOW-LET RADIATION DAMAGE IN HIGH-LET-LIKE SPATIAL STRUCTURE

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Microbeam experiments approximating high-LET tracks by bunches of lower-LET particles focussed to submicrometre scales (Schmid *et al*. 2012, Phys. Med. Biol. 57, 5889) provide an unprecedented benchmark for models of biological effects of radiation. PARTRAC track structure-based Monte Carlo simulations have verified that focussed 20 MeV proton bunches resemble the radial dose distributions of single 55 MeV carbon ions as used in the experiments. However, the predicted yields of double-strand break and short (<1 kbp) DNA fragments by focussed protons correspond to homogeneous proton irradiation and are much smaller than for carbon tracks. The calculated yields of dicentrics overestimate the effect of focussing but reproduce the fourfold difference between carbon ions and homogeneously distributed protons. The extent to which focussed low-LET particles approximate high-LET radiation is limited by the achievable focussing: submicrometre focussing of proton bunches cannot reproduce local nanometre clustering, i.e. DNA damage complexity characteristic of high-LET radiation.

INTRODUCTION

PARTRAC is a state-of-the-art tool for Monte Carlo simulations of radiation track structures, damage induction in cellular DNA and double-strand break (DSB) repair via the non-homologous end-joining (NHEJ) pathway[1]. Dedicated modules describe in an event-by-event manner the energy depositions by ionising particles in the traversed medium as well as the production and mutual reactions of reactive species. DNA damage is assessed by overlapping the track structures, namely energy deposition events for direct effects and distributions of radicals for indirect effects of radiation, with multi-scale chromatin models. The implemented DNA and chromatin structures range from atomic models of base pairs over nucleosomes, chromatin fibre, loops and domains to chromosome territories in eukaryotic cell nuclei. The NHEJ module represents the spatial mobility of individual DNA ends from the induced DSB and their enzymatic processing. Recently, this module has been extended to simulate chromosomal aberrations[2, 3].

PARTRAC calculations have been thoroughly tested against physical, chemical and biological data[1]. In recent microbeam experiments, protons at relatively low linear energy transfer (LET) values have been focussed to submicrometric scales to approximate high-LET tracks and their biological efficiency[4]. In these pioneering experiments, the methodology has been developed that enables separating the impact of the spatial distribution of initial DNA lesions from issues of lesion complexity on a local scale, which are in conventional studies on LET dependence inherently interlinked. Future experiments may also address temporal aspects by varying the particle fluence rate. These experiments thus provide data for a hitherto unprecedented advanced testing of the models and assumptions involved in Monte Carlo track structure-based biological models.

To this end, the scheme of radiation sources in PARTRAC has been upgraded to include irradiation with pre-defined matrices of ion bunches. First simulation results on the yields of DSB, DNA fragmentation patterns and dicentrics yields under conditions corresponding to the experimental set-up[4] are presented and discussed.

METHODS

Experimental data

In the experiments at the SNAKE microbeam[4], high-LET tracks have been mimicked by low-LET ions focussed to submicrometre scales. High-LET irradiation consisted in targeting single 55 MeV (estimated LET 310 keV μm^{-1}, Ref. [4]) carbon ions on a regular 5.4 μm grid. Focussed low-LET irradiation was realised by bunches of 117 protons at 20 MeV (2.65 keV μm^{-1} LET[4]) focussed onto a grid of the same size. The bunch profile was approximately Gaussian, with full width at half maximum (FWHM) estimated to lie between 0.3 and 1.0 μm. The given number of protons corresponds to the ratio of the carbon and proton LET values, so that the proton bunch delivered the same total dose as a single carbon track. Dose distribution profiles along the grid, calculated using an amorphous track structure model, indicated that the proton bunches

closely resemble the radial dose distribution of single carbon tracks except the extremely high energy deposition densities within carbon track cores. Moreover, a quasi-homogeneous proton irradiation pattern was used for comparison, in which four protons per quadratic grid element of 1 μm^2 were applied, yielding the same dose ($5.4 \times 5.4 \times 4 \approx 117$) of about 1.7 Gy.

As biological end points, micronuclei and dicentrics induction were reported for A_L cells. This is a hybrid cell line containing the standard set of Chinese hamster ovary chromosomes plus a single copy of human chromosome 11. For both micronuclei and dicentrics induction, focussed proton bunches were found significantly more efficient than homogeneously applied ones, yet considerably less effective than the high-LET carbon tracks.

Simulations with PARTRAC

The radiation source in the track structure module of PARTRAC has been upgraded to enable simulating the reported experimental set-up. In particular, the upgraded source scheme enables starting a defined number of Gaussian-distributed primary particles around a grid point, repeating this procedure for bunch centres on a regular grid and shifting this regular grid between two runs by a random displacement.

Tracks of single 55 MeV carbon ions on a 5×3 grid with 5.4 μm distance in x and y between grid points, 117 protons of 20 MeV focussed to various FWHM around these grid points, or quasi-homogeneously distributed protons on a 24×12 μm^2 source area with four particles per 1 μm^2 have been calculated with PARTRAC in 1000 runs representing 1000 irradiated cells. In each run, all ion tracks were exactly parallel with a small random deviation from the z-axis to avoid alignment effects with the principal axis of the modelled DNA and chromatin structures.

Interactions of the respective primary particles as well as of their secondaries have been simulated on an event-by-event basis in an 11-μm-thick target region, which included an ellipsoidal cell nucleus with 20 and 11 μm axis lengths and up to 6 μm thickness, covered by a 5-μm water layer. The A_L hybrid cell line used in the reported experiments has been approximated by chromatin structure derived for fibroblasts[1]. Details on cross sections, the simulation of direct and indirect radiation damages to DNA and parameters used can be found elsewhere[1].

Repair via the NHEJ pathway has been followed for the simulated DSB. Briefly, individual DNA ends from the DSB are considered independently. Their spatial mobility is simulated in parallel with the attachment of early NHEJ repair enzymes, formation of a synaptic complex of two DNA ends, and post-synaptic processing and final ligation. Dicentrics and other aberration types are scored from data on the origin of the joined fragments including the involvement of centromeres. Due to computing time constraints, only 100 runs were included in the calculation of repair and dicentrics formation. Model parameters derived from enzyme kinetics and DSB rejoining in fibroblasts as well as further details are discussed in Refs [1–3, 5, 6].

RESULTS

In Figure 1, the degree of focussing is illustrated, in terms of energy deposited to a voxel (10 nm × 100 nm × 200 nm in x, y and z) placed along the x-axis ($y = 0$). The particles impinge the region of interest in the z-direction, and the carbon tracks pass through the grid points ($x = \pm 2.7$ μm, $y = 0$) exactly, while these points represent the centres of the focussed proton bunches. Although averaging over 1000 particles, fluctuations follow from the fact that a single ionisation event (10.8 eV) in the given voxel volume corresponds to a specific energy imparted of about 8.6 Gy. The mean dose within the target volume was 1.81 and 1.85 Gy with a corresponding LET of 2.82 and 337 keV μm^{-1} for protons and carbon ions, respectively.

Homogeneously distributed protons and carbon ions produce on average 8.9 and 20.8 DSB per Gy per Gbp, respectively, in agreement with earlier results[7, 8] for these radiation qualities. For focussed protons, the yields are about 1 % higher. Another 1 % DSB fraction is linked to fragments shorter than 25 bp for all proton irradiations; the corresponding fraction for carbon ions is 16 %.

The simulated DNA fragmentation pattern is shown in Figure 2. For protons, the fragment yields below about 1 kbp length are independent of the focussing; they arise exclusively from single-track effects. Compared with

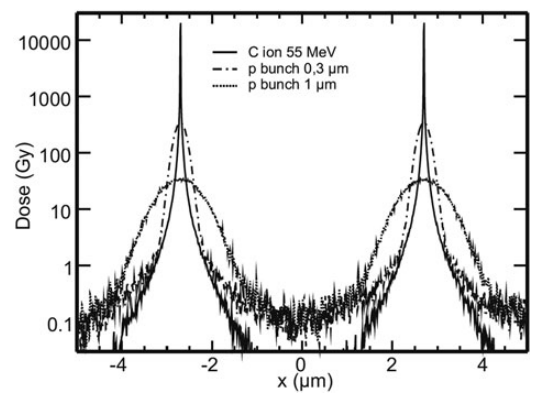

Figure 1. Dose as deposited by single 55 MeV carbon ions or by bunches of 117 20 MeV protons focussed to Gaussian profiles with FWHM of 1 and 0.3 μm, respectively, along the x-axis connecting two grid points (test volume of 10 nm ×100 nm × 200 nm). Results of 1000 simulations with PARTRAC are averaged.

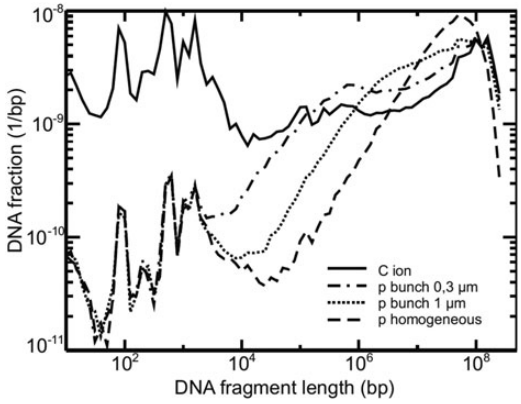

Figure 2. DNA fragment size distributions for the four irradiation schemes considered.

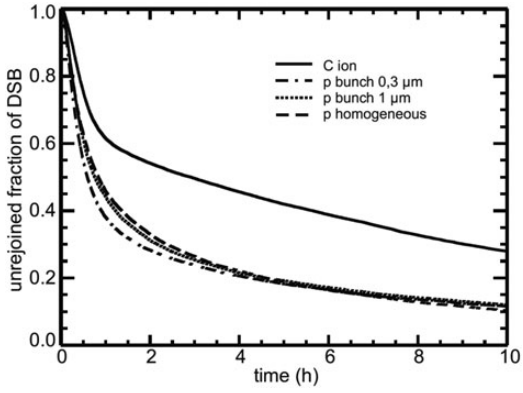

Figure 3. Predicted kinetics of DSB rejoining by NHEJ repair.

homogeneously distributed protons, DNA fragments around 100 kbp occur 4 and more than 10 times more frequently for proton bunches focussed to 1 and 0.3 µm, respectively. Both focussed proton bunches induce more Mbp-sized DNA fragments than C ions that, on the other hand, produce about 40 times more small fragments in the sub-kbp range, characteristic of high-LET ions[9]. Unfortunately, no experimental data on DNA fragmentation are available to directly test these predictions; however, the reliability of the predicted DNA fragmentation patterns for radiation of diverse quality has been shown previously[1,6].

The simulated kinetics of DSB repair by NHEJ is shown in Figure 3. The simulations predict the repair kinetics be almost independent of the focussing pattern for protons. For carbon ions, the slow repair component is dominant, in agreement with published experimental data[10]. In the model, the slow repair is assigned to the additional cleaning steps needed for

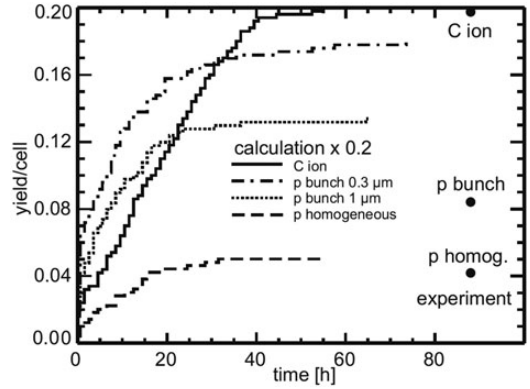

Figure 4. Simulated kinetics of dicentrics (yields scaled down by a factor of 5) compared with measured data from Ref. [4].

dirty DNA ends formed especially by high-LET radiations.

Predictions of the chromosome aberration module in PARTRAC for the induction of dicentrics by homogeneously distributed or focussed protons and by carbon ions are presented in Figure 4. The model directly links the aberrations with the underlying DSB (mis-)repair[2, 3]. As the slow component of repair is more pronounced for carbon ions, also the aberrations including dicentrics appear later than after proton irradiation. After 1–1.5 d post irradiation, the dicentrics yields by carbon irradiation are predicted to be higher than those by protons, even if the latter are focussed to the 0.3 µm FWHM. Only a very small fraction of DSB is still unrejoined even if the repair is followed for periods as long as 2 d, so that no further aberrations are formed after this time point. When scaling down the simulated results by a factor of 5 (for a discussion of this *ad hoc* factor, see Refs. [2, 3]), the predicted final yields of dicentrics correspond to the measured data[4] for carbon ions and homogeneously distributed protons (Figure 4). However, the dicentrics induced by focussed protons are still overestimated by factors of 2.1 and 1.5 for 0.3 and 1 µm FWHM, respectively.

DISCUSSION AND CONCLUSION

The PARTRAC biophysical modelling package has been upgraded to enable simulating focussed bunches of particles. Radiation tracks, induction of DNA damage, its repair and the formation of dicentric chromosome aberrations have been simulated for focussed protons with FWHM values corresponding to the lower and upper limits on the spot size and for homogeneously distributed protons and carbon ions as in the experiments.

The present simulations provide doses and LET values higher than the estimates reported by Schmid

et al.[4]. However, the difference amounts to about 7 and 9 % for protons and carbon ions, respectively, and corresponds to common variations between different radiation transport codes.

The yields of dicentrics are the only end point where the model results may be directly compared with the given experiment. Unfortunately, the present chromosome aberration model in PARTRAC has been found to overestimate the absolute yields of dicentrics, likely mainly due to overvaluing the long-distance mobility of DNA ends[2, 3]. Differences in the chromosome number and structure between the A_L hybrid cells and human fibroblasts may also contribute to the overestimation of absolute yields. The higher prediction for the focussing effect motivates careful appraisal of assumptions in the DNA repair model as well as in the chromatin model regarding intermingling of chromosome territories.

Generally speaking, the reported simulations support the idea that high-LET tracks can to some extent be mimicked by focussed bunches of low-LET particles. Outside the core of the carbon ion track, the dose distribution is rather similar to proton bunches with 0.3 μm FWHM; differences at larger distances where doses significantly below 1 Gy are deposited are of no concern for the effects analysed here.

On the other hand, initial DNA damage including its complexity on scales up to 1 kbp is the same for homogeneously distributed protons and focussed ones for FWHM values of at least 0.3 μm, but fundamentally different from carbon ions with their inherent characteristic patterns of high-LET radiation. This difference is highlighted in particular by the 40-fold yield of small DNA fragments. Thus, the complexity of DNA damage, i.e. the clustering of individual lesions, on a local scale such as that leading to DSB is given by track properties on nanometre scales like the core region of the carbon ion track. As focussing protons to bunches with FWHM on the order of a few nanometres is not feasible, the high complexity of carbon ion-induced clustered lesions, including total DSB or complex ones in particular, cannot be formed by focussed protons. Thus, the new experimental methodology[4] using submicrometre focussing of ions enables separating the spatial clustering of DNA lesions on the nm scale, i.e. DNA damage complexity, from that on larger scales of some 100 nm, whereas in conventional studies on LET-dependent radiation effects, issues of DNA damage complexity and its spatial distribution are inherently interlinked. Further investigations with focussed ions in combination with mechanistic modelling using PARTRAC and the local effect model[11] within the national project 'LET-Verbund' offer a hitherto unavailable tool for addressing the issue of radiation quality dependence of cellular radiation effects.

FUNDING

Supported by the project 'LET-Verbund' (Funding No. 02NUK031C) of the German Federal Ministry of Education and Research.

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