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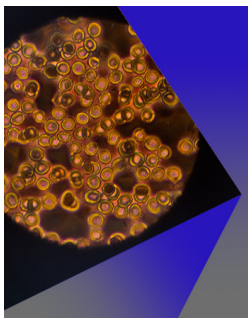

Biological effectiveness on live cells of laser driven protons at dose rates exceeding 10^9 Gy/s

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The ultrashort duration of laser-driven multi-MeV ion bursts offers the possibility of radiobiological studies at extremely high dose rates. Employing the TARANIS Terawatt laser at Queen's University, the effect of proton irradiation at MeV-range energies on live cells has been investigated at dose rates exceeding 10^9 Gy/s as a single exposure. A clonogenic assay showed consistent lethal effects on V-79 live cells, which, even at these dose rates, appear to be in line with previously published results employing conventional sources. A Relative Biological Effectiveness (RBE) of 1.4±0.2 at 10% survival is estimated from a comparison with a 225 kVp X-ray source. [http://dx.doi.org/10.1063/1.3699063]

I. INTRODUCTION

The use of ion beams in cancer radiotherapy exploits the advantageous energy deposition properties of ions as compared to more commonly used X-rays. Unlike X-rays, ions are able to deliver lethal amount of doses into the target tumour while limiting harm to the surrounding healthy tissues.[1] Hadron-therapy has been widely recognized across the globe and several clinical facilities, employing mainly protons from synchrotron, cyclotron or linac accelerators are operational and routinely treating a significant number of patients.[2] Unfortunately, the cost and size of these facilities, relative to megavoltage linac sources, are beyond the scope of a typical hospital and limit both the widespread use of proton treatments as well as employment of heavier ion beams. The idea of future facilities based on laser driven ion accelerators has been proposed as a way of reducing complexity and cost.[3–5] Significant effort is ongoing to demonstrate the ion beam parameters required to make this proposition viable. In the meanwhile, several groups have started preliminary work on the methodology and viability of using laser driven ion source for cell irradiation experiments.[6–8] The main aim of these investigations is to establish a procedure for cell handling, irradiation and dosimetry[9] compatible with a laser-plasma interaction environment. Furthermore, one of the peculiarities of laser-driven ion beams is their ultrashort duration, as ions are emitted in bursts of picosecond duration at the source and their therapeutic use may result in dose rates many orders of magnitude higher than normally used. The radiobiology at ultra-high dose rates is virtually unknown and warrants investigation of the biological effect of these ultrashort pulses at the cellular level.[10] In previous studies of dose-dependent cell damage employing ion bursts accelerated by fs laser systems, e.g. in Ref. 8, doses in the Gy range have been delivered to the cells in several fractions. Although each pulse delivered a fraction of a Gray in a short time (tens of ns), the average dose rate

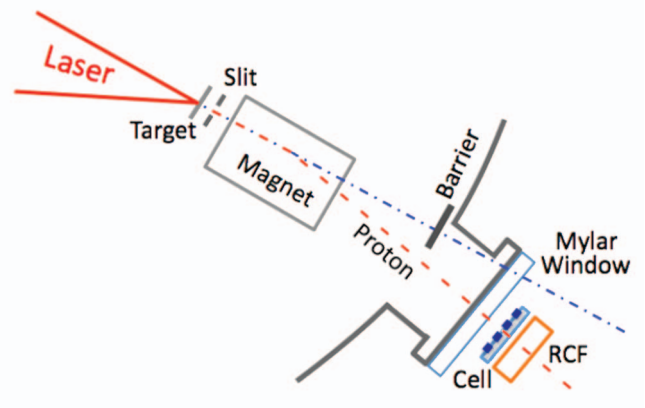

FIG. 1. Schematic of the experimental set-up. The blue and red lines represent the undeflected line of sight from the ion source to the cell plane and the trajectory of a deflected proton, respectively.

over a Gy-level exposure was in the Gy/s range, i.e. not dissimilar from the dose rate normally used in radiobiology with conventional proton sources.

II. EXPERIMENTAL METHOD

In this paper we report for the first time a quantitative study of dose-dependent biological effects of MeV protons on cells (V79 Chinese Hamster cell line) in the ultra-high dose-rate regime ($>10^9$Gy/s), with the dose (up to 5 Gy) delivered in a single exposure. The experiment was carried out at the Queen's University of Belfast (QUB) using the multi-Terawatt Chirped Pulse Amplified (CPA) laser system TARANIS.[11] This is a Ti:Sapphire-Nd:glass laser working at a wavelength of 1053 nm with a pulse duration of 700 fs and beam energy of up to 20 J. The laser was focused by a f/3 off-axis parabola (OAP) on 10 μm Al foils, leading to an intensity on target of order 10^{19} W/cm^2. In this intensity regime, the protons are created from the rear surface of the target by the well-known Target Normal Sheath Acceleration mechanism (TNSA),[12] which leads to broadband proton energy spectra, typically with particle numbers exponentially decreasing up to a cut-off energy.[13] The particles are typically emitted under a full-angle of 45° at 1-3 MeV energies, decreasing to ~10° degrees near the cut-off. As detailed in Ref. 13, ps laser-irradiation typically leads to high particle numbers, allowing for example number densities up to 10^{11}MeV^{-1}Sr^{-1} at 3 MeV.[11] This allows high doses to be attained in a single shot when irradiating a cell sample. An aperture slit 500 μm wide was located 10 mm behind the foil, and a dipole magnet (100 mm long, with a maximum field strength of 0.9 T) was placed 10 mm behind the slit in order to deflect the protons and disperse them according to their energies prior to the cell irradiation (see Fig. 1). Due to the relatively large entrance slit, each point was irradiated by protons within a certain energy range (see Fig. 2 for details).

The protons exited the vacuum chamber though a 50 μm thick Mylar window, which stopped protons with energies lower than 2 MeV. The distance from the window to the magnets output is 150 mm, with the cell dish placed 1cm from the window. The deflection angle of 10 MeV protons with respect to the direct source-aperture line-of-sight is about 14° (and larger for lower energies), which avoids cell-spot irradiation by unwanted X-ray radiation from the target collected through the aperture.

Customized Gafchromic films EBT2[14] (where the overlaminate and adhesive layers, normally preceding the active layer, were not present) were used directly behind the cell dish for dosimetry. The EBT2 films were previously calibrated using the cyclotron at the University of Birmingham. Prior to cell irradiation an experimental characterization of the proton spectrum at the cell locations was performed by EBT2 stack dosimetry.[15] Using a number of layers in a stack configuration it was possible at the same time to obtain the spatial proton energy distribution on the RCF films (i.e. the cell dots plane) and the energy deposition characteristics.

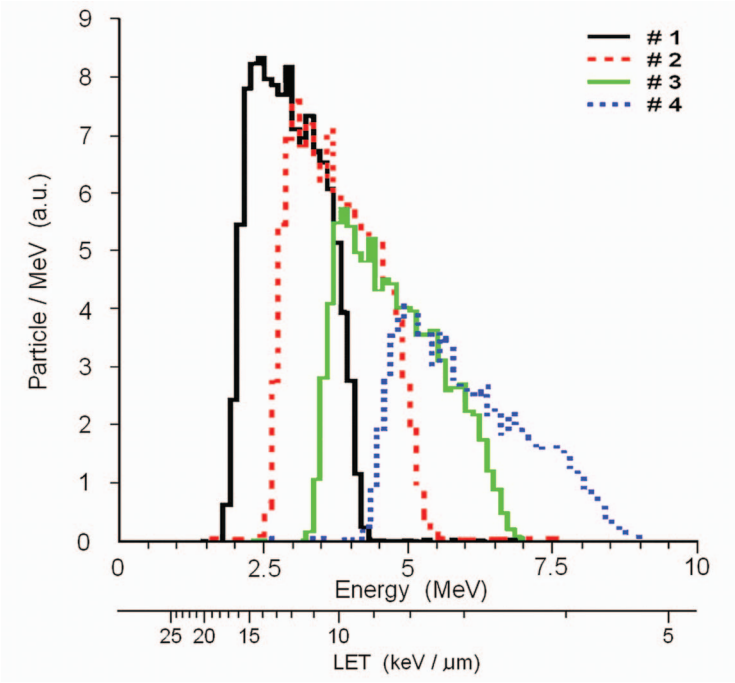

FIG. 2. Energy spectra of the protons (arbitrary units) delivered to the four cell spots in one of the configurations employed in the experiment. The entrance LET corresponding to the proton energies is also shown.

Monte-Carlo simulations of the proton propagation through the experimental configuration have been done using the FLUKA code.[16] By the simulation we can closely reproduce the proton spectrum we observe on the films and then reconstruct the number and spatial distribution of protons impacting on each cell-spot. Further details of the dosimetric procedure and of method employed to reconstruct the proton spectrum incident onto the cells can be found in Ref. 17. These energy characteristics result in a few ns duration of the proton bunch impacting on the cells.

The irradiation was performed on the radiosensitive Chinese hamster fibroblast cells (cell line V79), whose nuclei have dimensions of $\sim$10 μm in diameter and $\sim$ 9 μm in thickness.[18,19] The V79 cells were cultivated in Dulbecco's minimum essential medium (Lonza, UK) containing 10% FBS (Fetal Bovine Serum, Lonza, UK), 1% L-glutamine (Lonza, UK), and 1% penicillin/streptomycin (Invitrogen Ltd, UK). During any preparation of the cells, either pre-irradiation or post-irradiation, a laminar flow cabinet or a biosafety hood were used to avoid contamination from micro-organisms. The cells were seeded 10 hours before irradiation on a 175 μm thick Mylar sheet in order to give them time to multiply and attach to the substrate.

The cell "dots", i.e. disks of about 2.5 mm diameter, were then cut from the Mylar sheet and placed in a 50 mm diameter dish plate over a 3.5 μm Mylar film. Six disks were placed in each irradiated cell dish. Two disks were used as controls, which were not irradiated during the laser shot, while the proton irradiation was performed simultaneously on four cell dots, taking advantage of the proton energy spread across the cell plane. The position of the cell dots with respect to the proton distribution on the plane could be easily identified on the films, from the shadow projected by the Mylar disks. The dose released on the cell dots could be reconstructed directly by monitoring the RCF signal above and below the shadow of the disks. This approach allows us to target each dot with different doses ranging from a fraction of a Gy to a few Gys in a single irradiation, with individual assessment of the dose delivered to each cell dot. Spectral profiles within the different dots for one of the configurations used during the experiments are shown in Fig. 2. After the irradiation each cell dot was immersed into Trypsin to detach the cells from the Mylar support and then diluted in a prepared solution of medium and Trypsin. The cells were counted by Hemocytometer and a known

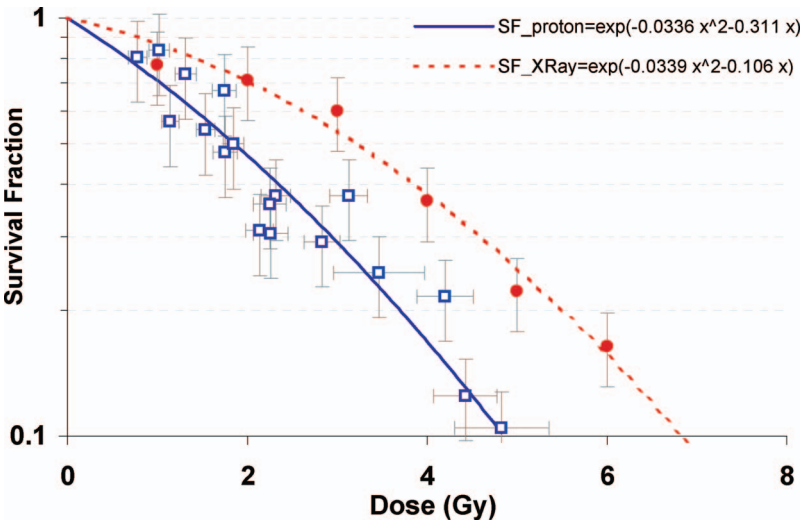

FIG. 3. Survival curve for the V79 cells obtained by irradiation with laser-accelerated proton bursts. The data are obtained using protons with energies in the range 1 - 5 MeV. A curve obtained by X-ray irradiation on the same cells, and using the same cell processing methodology, is also shown for comparison.

number of cells was plated into three Petri dishes. These were kept in an incubator in an atmosphere of 5% CO_2 and 95% humidity at 37°C for about 1 week.

In this environment, the cells which didn't experience lethal damage can start the process of repair post-irradiation and then multiply, forming cell colonies. After the incubation period the Petri dishes were stained with crystal violet to highlight the colonies, making them visible as violet dots, which can then be easily counted.

III. RESULT AND DISCUSSION

The Surviving Fractions (SF) were calculated as $SF = Num.\ of\ Colonies/(Num.\ of\ Cultured\ cell \times PE)$, where PE is the plating efficiency. Finally, for comparison, cell samples prepared according to the same procedure were irradiated with an X-ray source XRAD 225 kVp from PXI Inc. shielded by a 2 mm thick Cu filter to cut off the low energy part of the spectrum. Dosimetry for the X-ray exposures was performed using a Farmer chamber cross calibrated to a secondary standard detector.

The resulting survival curve is shown in Fig. 3, where the data has been fitted using a linear-quadratic model. The cells were irradiated with doses ranging from 0.8 to 5 Gy. The uncertainty in dose is mainly due to the finite size (2.5 mm) of the cell dots, while the error bars of the SF refer to the uncertainty associated with the method used in processing the cells and it is about 20% of the data point value.

The survival curve obtained with protons shows the expected higher biological efficiency of protons with respect to X-rays. From the comparison between proton and X-ray data, a Relative Biological Effectiveness (RBE) of 1.4±0.2 can be calculated at 10% SF. Direct comparison with results available in the literature is not straightforward, as the points in the curve are obtained using different cell dots irradiated at central energies in the range 1 - 5 MeV. However, the RBE value obtained is in the range of published values for MeV proton inactivation of V79 cells; in particular, Folkard *et al.*[20] obtain RBEs at 10% SF which range from 1.25 to 1.9 for proton energies (monochromatic) in the range 1-3.7 MeV, corresponding to Linear Energy Transfers (LET) of 10-30 keV/μm - a RBE of 1.4 is obtained for a LET of 17.8 keV/μm (1.8 MeV energies).

In Fig. 4, the points of the proton survival curve of Fig. 3 are grouped according to their LET. The LET for each of the proton data point is here considered as a volume averaged value on the cells, obtained using the energy spectrum associated with the corresponding cell dot, as done for example in Ref. 8. The Monte Carlo code SRIM[21] is used for simulating the energy deposited (LET) in the

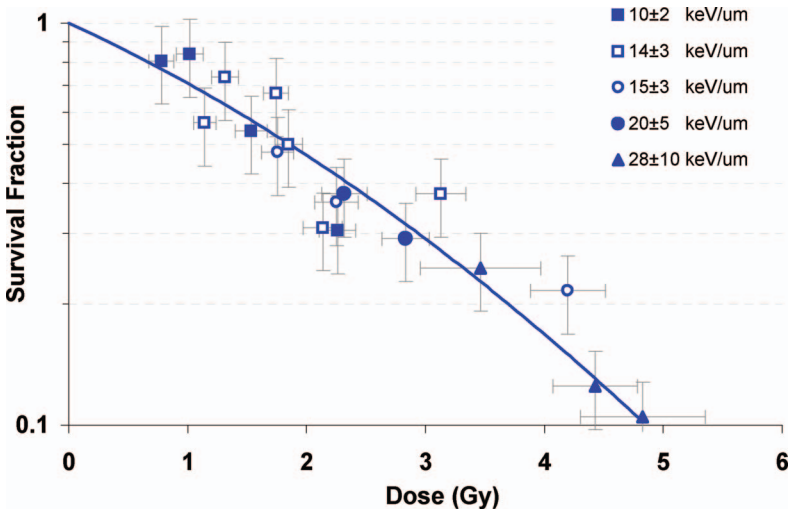

FIG. 4. Survival curve of Fig. 3, where the LET is indicated for each of the data points. The LET is a volume average over the cell monolayer, calculated by SRIM according to the spectra of the protons entering the cell dot.

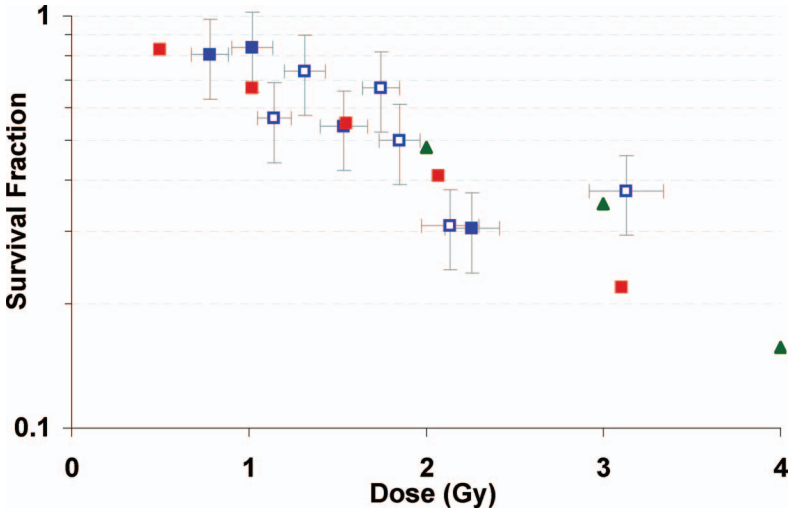

FIG. 5. Direct comparison between selected data from Fig. 3 and published data with comparable LET and proton energies. Solid blue squares have LET=10 keV/μm (central energy 5 MeV), open squares have LET= 14 keV/μm (central energy: 3.7 MeV). Solid red squares are data points from Ref. 20, obtained with protons of 3.7 MeV (10 keV/μm LET) (Reproduced with permission from, *Int. J. Radiat. Biol*, **69**, 729 (1996). Copyright 1996 Informa Healthcare). Green triangles are data points from Ref. 22, obtained with protons of 3.2 MeV (11 keV/μm LET) (Reproduced with permission from, *Int. J. Radiat. Biol*, **63**, 331 (1993). Copyright 1993 Informa Healthcare).

cell monolayer according to the proton spectrum, and the uncertainty in the LET is given by one standard error of the mean.

The survival rates observed are in line with published results obtained using conventional sources, as plotted in Fig. 5. In this figure SF data taken with peak energy of 3.7 MeV (14 keV/μm LET) and 5 MeV (10 keV/μm LET) are compared with data obtained at similar energy or similar LET using conventional proton irradiation of V79 cells.[20,22] The data indicates that, at the dose levels investigated, the ultrahigh dose rate employed has no significant effect on cell survival all-be-it that the cell handling methodologies were different.

This is an encouraging result in view of potential future therapeutic use of ultrashort bursts of laser-driven ions, as, in realistic arrangements, proton pulse durations on the cells will be of ns order or more, as employed in the measurements reported here. We note that similar conclusions

on independence of survival results from dose rate (although employing a different cell line and protons with different LET) have been very recently reached in experiments employing a pulsed ion microbeam,[23] operating at similar dose rates and pulse durations as in the experiment presented here.

We believe that, with an optimized set-up, it will be possible in the near future to increase the dose rate deliverable by laser-accelerated protons by a further 2 orders of magnitude, which will allow testing for the emergence of collective effects as predicted in Ref. 10.

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