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Measurement of relative biological effectiveness of protons in human cancer cells using a laser-driven quasimonoenergetic proton beamline

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Human cancer cells are irradiated by laser-driven quasimonoenergetic protons. Laser pulse intensities at the 5×10^{19} W/cm^2 level provide the source and acceleration field for protons that are subsequently transported by four energy-selective dipole magnets. The transport line delivers 2.25 MeV protons with an energy spread of 0.66 MeV and a bunch duration of 20 ns. The survival fraction of *in vitro* cells from a human salivary gland tumor is measured with a colony formation assay following proton irradiation at dose levels of up to 8 Gy, for which the single bunch dose rate is 1×10^7 Gy/s and the effective dose rate is 0.2 Gy/s for 1 Hz repetition of irradiation. Relative biological effectiveness at the 10% survival fraction is measured to be 1.20 ± 0.11 using protons with a linear energy transfer of 17.1 keV/μm. © *2011 American Institute of Physics*.
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Continued interest in laser-driven ion acceleration[1] is due to the potential for significant reduction in the size and cost of facilities[2] for ion beam radiotherapy (IBRT).[3] We demonstrated DNA double-strand breaking of human cancer cells by laser-driven proton bunches of short duration and high single bunch current.[4] Several investigations have subsequently reported radiobiological effects of high dose rate irradiation by laser-accelerated protons.[5–7] Experimental studies[4–6] to date have used protons with a large energy spread which is characteristic of laser-accelerated ions at the source (laser target). While it has been demonstrated that transport line optics downstream of the ion source is energy selective and can consequently deliver quasimonoenergetic beams,[8,9] such beamlines have not yet been applied to radiobiological studies. Quantitative evaluation of radiobiological effects critically require quasimonoenergetic irradiation.

We report the relative biological effectiveness (RBE) measurement for inactivation of human cancer cells using a laser-driven quasimonoenergetic proton beamline that consists of sets of miniature permanent magnets.

The J-KAREN Ti:sapphire laser system[10] at JAEA provided the intense laser pulses for target irradiation. The configuration of the irradiation system is seen in Fig. 1. Laser pulses of 1 J energy and 45 fs duration are focused to an intensity of $\sim5\times10^{19}$ onto a polyimide target foil of 7.5 μm thickness. Compatible with the 1 Hz laser repetition rate a new target area is provided for each pulse by advancing the foil tape with a servomotor. At the foil source the initial proton spectrum is continuous with a 4 MeV maximum kinetic energy.

The proton beamline consists of four dipole magnets, described by Luo *et al.*[11] in their design of therapy machine. Each dipole magnet consists of a pair of rectangular permanent magnets, generating a central magnetic field of 0.78 T. The second and third magnetic fields are parallel with each other and oriented antiparallel to the first and fourth ones. Protons are collimated by an entrance pinhole and laterally displaced from the target normal axis in the midplane (midway between the second and third magnets) by the first two magnets. Proton energy and energy spread are set by a movable 5 mm diameter pinhole that is located in this midplane. The final two magnets steer protons back to the target normal axis. The transverse beam profile is adjusted by a pinhole. Protons are finally extracted from vacuum into air through a thin polyimide window of 7.5 μm thickness and 10 mm diameter.

As seen in Fig. 1, the capsule of cell samples is located close to the vacuum window. Cell samples are cultured on a polyimide cell dish of 7.5 μm thickness at the bottom of the capsule. To irradiate these cells protons must pass through the first 7.5-μm-thick polyimide vacuum window, 3 mm of laboratory air, and the 7.5-μm-thick cell dish, keeping their kinetic energy to be high enough to penetrate the cell monolayer. Details of the capsule structure and cell culture are described elsewhere.[12]

The proton-energy spectrum transported through the beamline is measured with online single bunch time-of-flight (TOF) spectrometry.[13] The TOF detector is a plastic scintillator (42 mm in diameter) with an upstream 5 mm diameter collimator to duplicate the cell capsule aperture. Figure 2(a) shows the tunable quasimonoenergetic proton spectra trans-

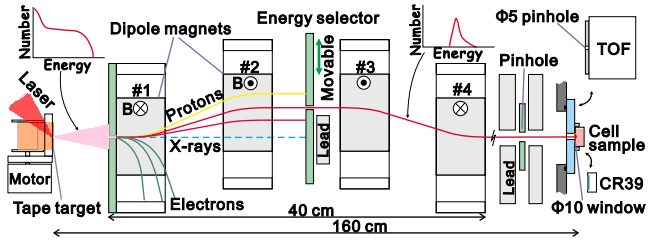

FIG. 1. (Color online) Experimental setup of the laser-driven *quasimonoenergetic* proton beamline.

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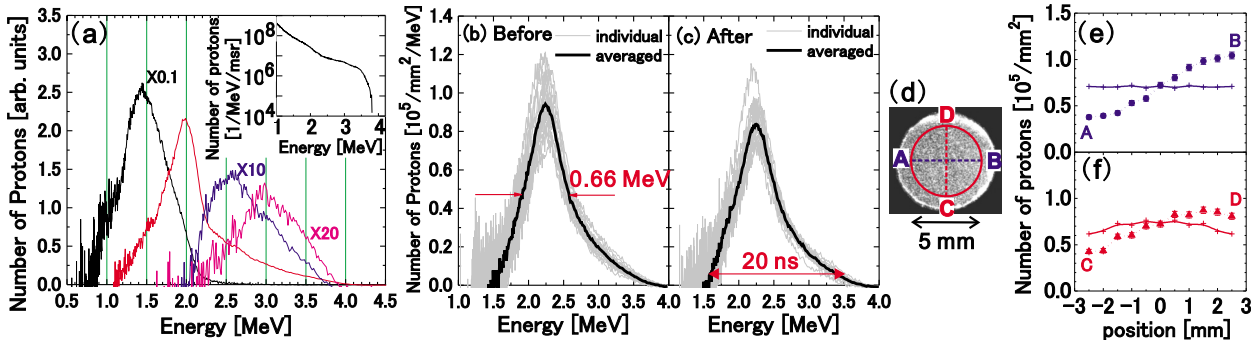

FIG. 2. (Color online) (a) Results of beam-energy selection by the four dipole magnets observed with a TOF spectrometer for different energy tunes. The inset shows the proton-energy spectrum obtained without the dipole magnets. (b) and (c) Energy spectra of protons monitored before and after cell irradiation. (d) A beam-spot image near the cell sample position (circle) measured with CR-39. (e) and (f) A proton areal densities along the axes A-B and C-D, respectively, of (d). The proton distributions used for the cell irradiation using the 180° cell rotation are shown with solid lines.

ported through the beamline. The central energy could be tuned from 1.5 to 3 MeV by moving the midplane pinhole position laterally between 22 and 10 mm from the target normal axis. 2.25 MeV protons were used for cell irradiation.

To assess the shot-to-shot fluctuation of single bunch current we recorded TOF spectra for 20 successive laser shots before and after cell irradiation on a single day as seen in Figs. 2(b) and 2(c), respectively (single-shot spectra are individually shown in gray and the averaged spectrum is displayed in black). The shot-to-shot reproducibility of spectral shape is reasonable at 1 Hz. The energy spread of the averaged spectrum is 0.66 MeV [full width at half maximum (FWHM)]. The variations (standard deviation, σ_{fluc}) of proton number over 20 shots are 12.9% and 12.3% in Figs. 2(b) and 2(c), respectively. This is attributed to the short term stability of the laser intensity. Over the duration of one measurement cycle (that included irradiation of 14 samples and CR-39 measurements) the proton number of the averaged spectra of Figs. 2(b) and 2(c) reveals a longer term drift of 10.5%.

The areal distribution of protons was observed before and after cell irradiation with CR-39 track detector film alternatively placed at the cell sample position. Figure 2(d) displays a CR-39 image (after KOH chemical etching) indicating the proton distribution. The large circular white region, whose diameter matches that of the cell capsule aperture, is induced by proton bombardment. Cancer cells were located at the center of this proton irradiation field as indicated by the circle (5 mm in diameter) in the figure. The areal distribution profile is determined by counting the number of proton-induced etch pits (tracks) with a microscope along the horizontal (A-B) and vertical (C-D) lines of Fig. 2(d). The distribution of proton areal density is displayed as dots in Figs. 3(e) and 3(f) in units of $10^5/\mathrm{mm}^2$ for the A-B and C-D lines, respectively. The nonuniform areal proton density distribution is observed as a tilt. After the first 10 of 20 shots the cell capsule was therefore rotated by 180° to reduce this nonuniformity. A more uniform density distribution (σ_{area}=8.0%) was achieved by the rotation as seen in the solid lines of Figs. 3(e) and 3(f) for the A-B and C-D lines, respectively.

The absorbed dose D integrated over n bunches is determined by the following equation:

$$D(\mathrm{Gy}) = n \int_{\mathcal{E}_0} d\mathcal{E}_0 \cdot \frac{C \cdot N(\mathcal{E}_0) \cdot E_d(\mathcal{E}_0)}{Q \Delta x} \cdot 1.602 \times 10^{-7}. \quad (1)$$

Here, $C=7.20\times10^4$ mm^{-2}: the averaged density of proton number cross-checked by TOF and CR-39 detectors, $Q=1$ g/cm^3: the mass density of liquid water, and $\Delta x=5$ μm: the thickness of the cell monolayer. $\mathcal{E}_0$ is the proton energy in vacuum (i.e., before entering the thin-foil window) and $N(\mathcal{E}_0)$ is the normalized energy distribution of protons satisfying $\int N(\mathcal{E}_0)d\mathcal{E}_0=1$. We determine $N(\mathcal{E}_0)$ from the averaged TOF spectrum seen in Figs. 2(b) and 2(c). $E_d(\mathcal{E}_0)$ is the energy that is deposited in the cell layer (in keV units) by protons with energy $\mathcal{E}_0$. The dynamics of energy deposition are simulated with the three-dimensional (3D) Monte Carlo TRIM code.[14] We calculate the energy loss of protons in a multilayer target consisting of the 7.5-μm-thick polyimide window, 3 mm of air, the 7.5-μm-thick polyimide cell dish, and 5 μm of liquid water (assumed to be equivalent to the cell monolayer). Typically, protons of $\mathcal{E}_0=2.25$ MeV are decelerated down to 1.9 MeV at the entrance of cell layer. From Eq. (1), the single bunch dose is estimated to be 0.2 Gy, corresponding to a single bunch dose rate of 10^7 Gy/s. At the 1 Hz repetition rate this amounts to a duty factor of 2×10^{-8} with an average dose rate of 0.2 Gy/s. We estimate

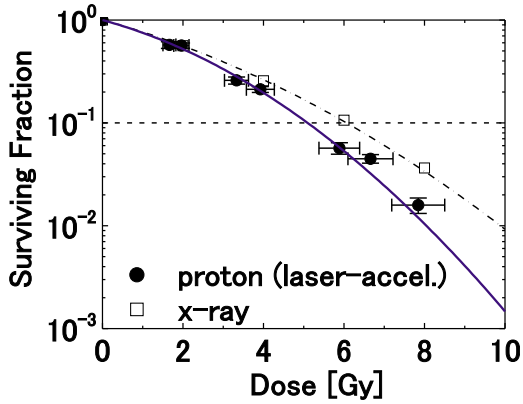

FIG. 3. (Color online) The fraction of surviving cells after the irradiation with the laser-accelerated protons (closed symbols) and a reference x ray (open symbols) as a function of dose. For the proton data, the statistical error ΔD is indicated by horizontal bars.

the statistical error of the integrated proton dose (n shots) accordingly to be

$$\Delta D = D\sqrt{(\sigma_{fluc}^2 + \delta^2)/n + \sigma_{area}^2}, \quad (2)$$

where the first standard deviation, σ_{fluc} (12.9%), is the shot-to-shot fluctuation of proton number, the second one, δ (10.5%), is its long term drift, and the third one, σ_{area} (8.0%), is the areal proton density fluctuation.

The proton linear energy transfer (LET) in the cell monolayer is evaluated with the 3D TRIM code. We obtain a volume-averaged LET which is $\int_{\mathcal{E}_0} d\mathcal{E}_0 N(\mathcal{E}_0)\cdot E_d(\mathcal{E}_0)/\Delta x$. Taking into account the proton-energy spread of 0.66 MeV (FWHM), the LET is determined to be 17.1 ± 2.8 keV/μm.

Using a colony formation assay, we have determined the RBE value for cell inactivation by laser-accelerated ions. Figure 3 shows the fraction of surviving cells after the irradiation with the laser-accelerated protons (closed symbols) and a reference x-ray (open symbols) as a function of dose D up to 8 Gy. A 4 MV clinical linac at HIBMC provided the x-ray irradiation.[15] Cell processing and handling details are described elsewhere.[12] The data obtained are analyzed according to linear-quadratic model,[16] where the surviving fraction (SF) is described by the equation, $\mathrm{SF}=\exp(-\alpha D - \beta D^2)$. By the curve fits with least-squares method, we determine the parameter values of $\alpha = 0.243 \pm 0.027$ and $\beta = 0.0409 \pm 0.0091$ for the proton data and $\alpha = 0.244 \pm 0.006$ and $\beta = 0.0224 \pm 0.0017$ for the reference x-ray data. In Fig. 3, the fit curves are shown with solid (proton) and dotted (x-ray) lines. RBE is evaluated from the dose at the 10% surviving fraction, $D_{10}(p) = 5.06$ Gy for protons and $D_{10}(\mathrm{x}) = 6.06$ Gy for x rays. Including errors on the proton dose, we determine the RBE value to be $\mathrm{RBE} = D_{10}(\mathrm{x})/D_{10}(p) = 1.20 \pm 0.11$ for the laser-accelerated protons with a volume-averaged LET of 17.1 ± 2.8 keV/μm, mentioned above.

Using a conventional accelerator, Folkard *et al.*[17] obtained a RBE value of 1.4 ± 0.2 for hamster cells (V79) irradiated by protons with a similar LET of 17.8 keV/μm (1.83 MeV in energy). Although it is important to consider the difference between the cell lines, the two RBE values are nonetheless comparable. In this LET regime, with the average dose rate comparable to that of conventional accelerator sources (0.2 Gy/s with 1 Hz operation) we observe no significant effect of the very high single bunch dose rate.

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