DNA DOUBLE-STRAND BREAK INDUCTION IN A549 CELLS WITH A SINGLE-BUNCH BEAM OF LASER-ACCELERATED PROTONS

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Abstract

Laser-acceleration of ions has been suggested as a potential basis for technology that is alternative to conventional accelerators for ion beam radiotherapy (IBRT). To investigate the effects of high dose rates that are attributed to high current, short bunch irradiation we have developed an experimental setup that uses laser-accelerated protons. In-vitro human lung cancer cells: A549 pulmonary adenocarcinoma are irradiated with laser-accelerated proton bunches with a duration of 2×10^{-8} s and flux of $\sim 10^{15}$ cm^{-2}s^{-1}, amounting to single bunch absorbed dose at the 1 Gy level. The double-strand break (DSB) yield in cell DNA is analyzed using the phosphorylated histone H2AX immunostaining method.

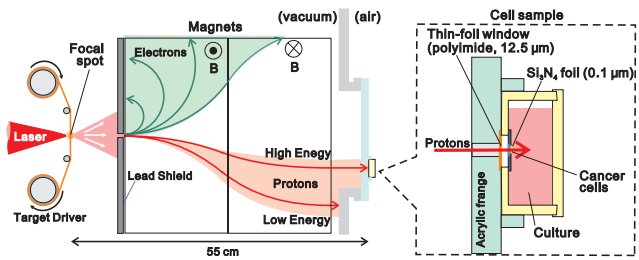

Figure 1: Side view of experimental setup of the laser-driven short-bunch proton irradiation apparatus, indicating proton trajectory to the cell sample: MeV-proton source, energy-selection magnetic dipole pair and a culture capsule of cell samples.

INTRODUCTION

It has been widely recognized that the use of particle ion beams in cancer radiotherapy has the physical advantage of delivering longitudinally a more localized dose deposition associated with the well-known Bragg peak phenomenon [1].To date, more than 52,000 patients have been treated by ion beams at 25 institutes all around the world. However, the high capital cost of IBRT facilities remains a primary hurdle to a more widespread access to this treatment modality.

Recently, high-intensity lasers have been suggested as a potential, cost-saving alternative [2] to conventional ion accelerators for the radiotherapy. When a laser pulse with intensity well exceeding 10^{18} W/cm^2 interacts with a foil target, the laser field accelerates a significant number of electrons to relativistic velocities. Some of these 'hot' electrons pass through the foil generating a strong electrostatic field exceeding 1 TV/m, at the rear (downstream) surface, which can surpass the ion-acceleration field typical of conventional accelerators by six orders of magnitude.

A unique feature of laser acceleration is the extremely-high peak current attributed mostly to the short duration of a single proton bunch. In recent works [3], single high-intensity laser pulses have produced proton bunches of charge level, 10^{11}, corresponding to $\sim$ 1 kA peak ion currents 1 mm from the target. However, there has been no experimental work investigating biological effects of such high-current, short-bunch laser-driven ion beams.

To address the fundamental question we had developed a laser-driven ion irradiation apparatus [4]. Here we report that we have successfully increased the single bunch intensity and proton absorbed dose by one order of magnitude compared to that in the previous work [4]. This system improvement enables single bunch 1-Gy proton irradiation over a 20-ns duration. We report the experimental results of observed DNA-DSBs in human cancer cells obtained with this higher irradiance setup.

DEVELOPMENTS AND EXPERIMENTS

A schematic drawing of the setup is shown in Fig. 1. The apparatus is divided into three main system components: (i) a high-intensity laser system, J-KAREN (not shown in Fig. 1), (ii) a target which is the MeV proton source when irradiated by a focused laser pulse and (iii) a permanent magnet spectrometer for the proton beam energy selection.

J-KAREN is a femtosecond high-intensity laser system that combines both Ti:sapphire chirped pulse amplification

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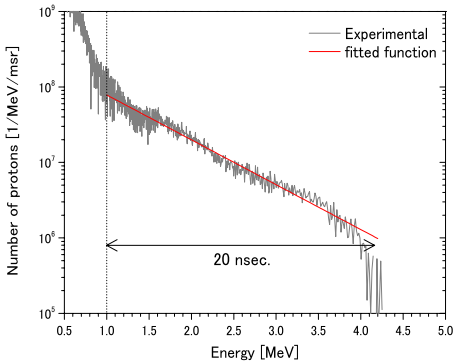

Figure 2: A typical energy spectrum (dotted line) of laser-accelerated protons measured in vacuum with Time-of-Flight (TOF) spectrometry. The number of protons is shown in unit of 1/MeV/millisteradian (msr) as a function of the proton energy $\mathcal{E}_0$ in MeV. A fit to the exponential slope indicates a characteristic temperature, $k_BT = 0.73$ MeV.

(CPA) and optical parametric CPA (OPCPA) techniques [7]. The system consists of two successive CPA stages, where the second one includes a three-pass Ti:sapphire final booster amplifier pumped with the second harmonic from a high-energy Nd:glass laser. For the present study, laser pulses of 1.8 J are delivered to the experimental chamber with a repetition rate of 1 shot/15 min. This 15-min. interval is necessary for cooling of the glass pump laser system.

High power laser light is focused by an off-axis parabolic mirror (OAP) at onto a target at a 45° incident angle with a typical laser-spot diameter of $\sim$ 5 μm, resulting in the peak laser intensity of 7×10^{19} W/cm^2. Then, the laser accelerates electrons at the target surface up to relativistic velocity resulting in a highly-concentrated flow of electrons through the target and toward its rear side, producing a strong charge-separation electric field in a plasma cloud. This plasma acts as both an ion source as well as a *micron-sized* accelerator for MeV-energy proton generation. Energy spectra of the accelerated protons are measured downstream of the source in vacuum by a Time-of-Flight (TOF) spectrometry [5] (Fig. 2) before the biological irradiation is conducted.

Protons are accelerated from the rear target surface at a typically large divergence angle of order $\sim 10°$. In this case a new target site (12.5-μm-thick polyimide tape) is positioned at the laser focus for each laser shot by a servo-motor that continuously drives the tape. The proton beam is separated by a 1-mm-wide slit in lead plate for x-ray shielding and introduced into the energy selector [4] for the protons. This system consists of a pair of dipole magnets with antiparallel magnetic fields of magnitude 0.04 T. Therefore high-energy co-moving electrons, which are ejected from the target simultaneously with protons, are steered in a direction opposite to that of protons and efficiently removed in the first dipole magnet. On the other hand, protons are slightly steered by the first magnetic field, and the other way by the second one, such that transmitted proton trajectories are laterally shifted from the target-normal axis (beamline), by an energy-dependent displacement. Protons of energy below 1 MeV, which cannot penetrate the foil window mentioned above, are removed by the magnetic system, as described in ref. [4]; hence, only the higher-energy proton component of the spectrum ($\mathcal{E}_0 > 1$ MeV) irradiates the sample capsule located downstream of the magnetic dipole pair. With TOF spectrometry we determine that proton bunch duration, with an energy spectrum ranging from 1 to 4.2 MeV, is 20 ns at the capsule position.

After passing through the energy selecting dipole pair protons are transported into air through a thin-foil vacuum window made of a polyimide foil of 12.5 micron thickness (the same as that used for the tape target). As depicted in Fig. 1(b), the capsule of cell samples is located close to the vacuum window. The capsule has a 0.1-μm-thick cell dish made of a silicon nitride (Si_3N_4) foil. The cell samples are cultured directly on the surface of the cell dish attached to the bottom of the capsule. Consequently protons irradiate cells after passing through the first 12.5-μm-thick polyimide vacuum window, 3-mm of laboratory air plus the 0.1-μm-thick cell dish, keeping their kinetic energy to be high enough at the cell sample.

In this work, we used the human lung cancer cell line: A549 pulmonary adenocarcinoma as a sample. A549 was cultured with 10% fetal bovine serum (Biowest) and 0.025% penicillin/ streptomycin (Gibco) containing Dulbecco's Modified Eagle Medium (Nihon pharmaceutical) in 5% CO_2 and 95% air. A day before the irradiation, an adequate number of cells were seeded on the cell dish. The average dimensions of a cell nucleus were measured to be a width near $\sim$ 20 μm and thickness near $\sim$ 5 μm by a high-resolution laser-probe microscope (Keyence VK-9700 Generation II).

RESULTS AND DISCUSSION

In order to estimate absorbed dose of the protons, we have measured areal distribution of protons at the exact position at the cell location. Before and after the cell irradiation, we inserted a piece of CR-39 nuclear track detector (BARYOTRACK-P, Nagase Landaur ltd.) on the bottom of the capsule, *i.e.*, on the Si_3N_4 thin foil. Protons bombard the CR-39 plate after traversing the same path and materials as in the cell-irradiation case (through the polyimide-foil window, air and the Si_3N_4 foil). Incident protons are detected as the latent tracks left in the CR-39, in the form of broken molecular chains and free radicals that are revealed after etching with a caustic alkali solution. As a result, the average proton areal density per bunch is measured to be $C = 2.11 \times 10^5$ mm^{-2}. The typical size of an A549 nucleus (20 μm in diameter) is represented by the dashed circle in the image. Consequently, each A549 nucleus is irradiated with an average of about 70 protons during the short duration of a single bunch.

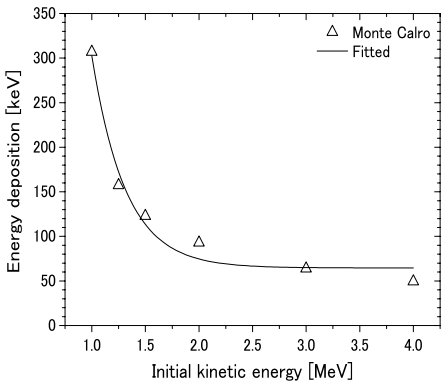

Figure 3: Proton energy loss the cell layer $E_{\rm d}$ calculated with eq. 1.

The dynamics of proton collisions are simulated with the 3-dimensional Monte-Carlo TRIM code[6]. We calculate the energy loss of protons $d\mathcal{E}/dx$ in a multi-layer target consisting of a 12.5-μm-thick polyimide, 3-mm of air, 0.1-μm of Si_3N_4 and 5-μm of liquid water, which is assumed to be equivalent to the layer of cancer cells planted on the Si_3N_4 foil.

By integrating the $d\mathcal{E}/dx$ along the depth x, the energy deposited in the cell layer $E_{\rm d}$ is determined for each incident proton energy $\mathcal{E}_0$ according to:

$$E_{\rm d}(\mathcal{E}_0) = \int_x \frac{d\mathcal{E}}{dx} dx. \qquad (1)$$

Here, $d\mathcal{E}/dx$ is in units of keV/μm and integrated over the cell layer. The $\mathcal{E}_0$ dependence of $E_{\rm d}$ is displayed in figure 3. For convenience in absorbed dose estimation this result can be fit to the following functional form:

$$E_{\rm d}(\mathcal{E}_0) = 5.64 \times 10^3 \cdot \exp(-\frac{\mathcal{E}_0}{0.316}) + 64.6 \qquad (2)$$

and the absorbed dose D in units of Gy in determined as follows:

$$D = \int_{1.0}^{4.2} d\mathcal{E}_0 \cdot \frac{C \cdot N(\mathcal{E}_0) \cdot E_{\rm d}(\mathcal{E}_0)}{Q\Delta x} \cdot 1.602 \times 10^{-7}. \qquad (3)$$

Here, Q is the mass density of liquid water in g/cm^3, and $\Delta x = 5$ μm is the thickness of the cell layer. Recall that the proton areal density, C is 2.11×10^5 mm^{-2} and the normalized energy distribution can be expressed as$N(\mathcal{E}_0) = 5.53\exp(-\mathcal{E}_0/0.73)$ (see Fig. 2). Accordingly (using equation (5)) we determine the absorbed dose of protons, D to be $\simeq 1$ Gy from a single proton bunch.

Induction of DNA double strand breaks (DSB) in A549 cells is investigated using phosphorylated histone H2AX immunostaining method [8]. It has been recognized that the presence of H2AX phosphorylation along a DNA strand corresponds only to a DNA DSB site. Therefore, the manifestation of phosphorylated H2AX in the nuclei (termed as γ-H2AX focus formation) can be used as a criterion for a DNA DSB.

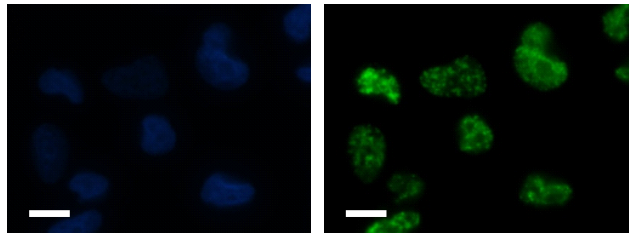

Figure 4: Typical results of γ-H2AX immunofluorescence microscopy obtained for the laser-accelerated proton irradiations of 1 Gy. Bar, 10μm.

A typical result of γ-H2AX immunofluorescence staining is shown in Fig. 4: Nuclei (left) and γ-H2AX foci (right) stained with blue and green, respectively. It should be emphasized that this result is obtained with a single proton bunch of 20 nanosecond duration. Here, the A549 cells were incubated at 37 °C for 30 minutes with CO_2 incubator after the proton dose of 1 Gy. The cells were subsequently fixed with 95% methanol for 10 minutes, rinsed three times in a phosphate buffer saline, and blocked with 20% bovine serum albumin. For immnofluorescence staining of γ-H2AX and nuclear staining, Alexa Fluor 488 conjugated anti-γ-H2AX antibody (♯9719, Cell Signaling Technology, MA, Denver) and DAPI (4', 6-diamino-2-phenylindole, Fluka) were used, respectively.

By counting the number of γ-H2AX foci apparent on the microscopic images, we estimate the DSB yield to be 41.3 DSB/nucleus for the absorbed doses of 1 Gy, respectively. As a comparison, DSB yield is obtained for 1.71 MeV x-ray irradiation (average energy) delivered from a 4-MV linac, resulting in 28.1 DSB/nucleus for 1 Gy. The relative biological effectiveness (RBE) value can be determined as the ratio of the DSB yields for laser-accelerated protons to that of the x-rays. Corresponding value of RBE are obtained for the 1-Gy irradiation to be 1.47, which is the first one estimated for the single 20 nanosecond proton bunch irradiation.

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