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Application of laser-accelerated protons to the demonstration of DNA double-strand breaks in human cancer cells

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We report the demonstrated irradiation effect of laser-accelerated protons on human cancer cells. *In vitro* (living) A549 cells are irradiated with quasimonoenergetic proton bunches of 0.8–2.4 MeV with a single bunch duration of 15 ns. Irradiation with the proton dose of 20 Gy results in a distinct formation of γ-H2AX foci as an indicator of DNA double-strand breaks generated in the cancer cells. This is a pioneering result that points to future investigations of the radiobiological effects of laser-driven ion beams. Unique high-current and short-bunch features make laser-driven proton bunches an excitation source for time-resolved determination of radical yields. © *2009 American Institute of Physics*. [DOI: 10.1063/1.3126452]

After the initial proposal by Wilson,[1] it has been widely recognized that the use of particle ion beams in cancer radiotherapy has the physical advantage of delivering highly local dose distributions due to the well-known inherent Bragg peak phenomenon of energy deposition. Further benefit of the ion beam therapy (IBT) was found with increased relative biological effectiveness (RBE) within the Bragg peak region.[2]

Recently, high-intensity lasers have been suggested as a potential cost-saving alternative[3] 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,[4–6] 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,[7,8] single high-intensity laser pulses have produced proton bunches of charge level at 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.

In this letter, we describe a laser-driven ion irradiation apparatus for biological studies. We demonstrate DNA double-strand breaks (DSB) of *in vitro* human cancer cells and discuss the potential of the laser-driven ion beam as a short-pulse excitation source for biochemical reactions.

The experiment was performed using the J-KAREN (Ref. 9) Ti:sapphire laser system at JAEA. The laser pulses of 0.6 J energy and 35 fs duration are focused to a peak intensity of 5×10^{19} W/cm^2 onto a thin foil target, which is continuously fed by a servomotor, providing a "fresh" target surface for each laser shot. The laser pulses are delivered at a repetition rate of 1 Hz. As illustrated in Fig. 1(a), such intense laser irradiation generates energetic protons that diverge from the rear side of the target with a half-cone angle of $\sim$10° with respect to the normal of the foil rear surface. The proton beam has a continuous distribution of the energy up to 2.5 MeV, as illustrated in Fig. 2(a) with a gray line.

Accompanying proton emission, are electrons and x rays that are generated simultaneously from the laser-induced plasma. In order to remove them, we used a pair of dipole magnets with magnetic fields of 0.04 T oriented antiparallel
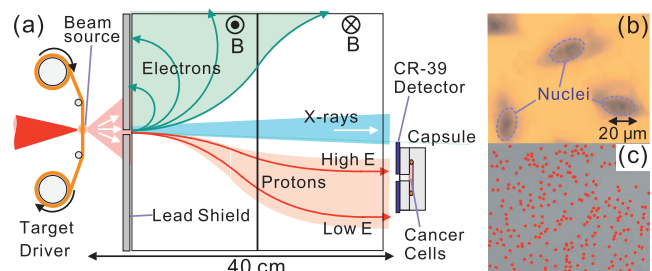

FIG. 1. (Color) (a) A schematic drawing of experimental setup. (b) An image of cancer cells taken by a microscope. (c) A spatial distribution of protons detected by CR-39 in a single laser shot. Each red point represents a single proton bombardment. The screen size is set to be same as that in the frame (b).

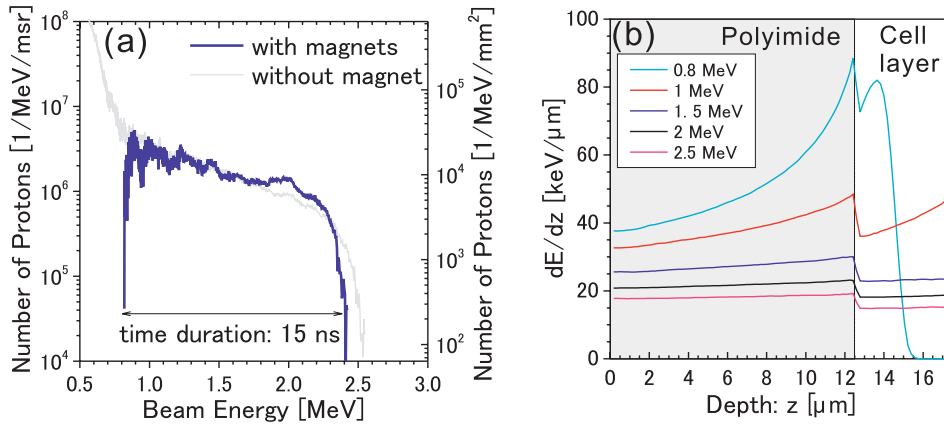a)Electronic mail: yogo.akifumi@jaea.go.jp.

FIG. 2. (Color) (a) Typical energy spectra of proton beams obtained with the magnetic fields (blue line) and without the magnetic fields (gray line). Each spectrum shows the number of protons obtained in a single laser shot. (b) The results of 3D Monte Carlo simulations: distributions of electronic energy loss $d\mathcal{E}/dz$ as a function of the depth z.

to each other, as shown in Fig. 1(a). Protons, electrons and x rays enter the first magnetic field through a 1-mm-wide one-dimensional aperture made on a lead shield. Protons are steered in the opposite direction to that of the electrons, while x rays propagate straight through along the target normal axis. In the present experiment, electron energies are observed to be lower than 2 MeV, corresponding to a Larmor radius of about 20 cm; meaning that they are completely swept away from the normal axis and the protons. On the other hand, the proton trajectory is steered slightly by the first magnetic field, and again by the second one, such that transmitted proton trajectories are laterally displaced from the target normal axis by an energy-dependent distance.

The experimental setup as shown in Fig. 1 is placed in a vacuum chamber and the irradiated cancer cell culture is enclosed in a specially designed capsule. As shown in Fig. 1(a), the capsule consists of a vessel for culture solution and a 12.5-μm-thick polyimide foil window for protons. The foil window and the vessel are sealed by a silicon rubber o-ring that is fixed by screws with a lid having a pinhole of 2 mm in diameter on its center. We use human lung cancer cells: A549 pulmonary adenocarcinoma, the microscopic image of which is shown in Fig. 1(b). The average cell nucleus is of width ~20 μm and thickness of ~5 μm, as determined by a laser-probe microscope (Keyence VK-9700 Generation II). The *in vitro* A549 cells are cultured directly on the surface of the polyimide foil. Protons irradiate the cells after penetrating the polyimide film. The polyimide foil is strong enough to sustain a 1 atm pressure difference across it (the culture solution is at 1 atm). During the proton irradiation, we placed the capsule at the exit of the magnet pair, shifting the capsule window by 5 mm from the center axis of the magnets. Consequently only higher-energy protons enter the capsule window and irradiate the cells, as shown in Fig. 1(a). X rays emitted from the target emerging around the center axis are cut off by the lead shields. They do not radiate the cell samples.

The energy and number of protons are determined by a time-of-flight spectrometer[10] located downstream of the magnets, with the capsule temporarily removed and a different 2-mm-wide aperture placed at the same position as the capsule window. A typical incident energy spectrum obtained at this location is shown as a blue line in Fig. 2(a). The figure clearly shows that the energy spectrum looses its lowest-energy components ($<$0.8 MeV, as seen in the gray line) when the magnets are used. The proton bunch duration at the capsule position is about 15 ns. Protons with the energies

above 0.8 MeV can penetrate the polyimide window to irradiate cancer cells. The measured number of protons per bunch varies by less than 20% (bunch-to-bunch) at the 1 Hz repetitive rate.

The number of protons corroborated during cell irradiation by an ion-track detector (CR-39), which is covered with another 12.5-μm-thick polyimide foil and placed beside the capsule window. Protons penetrating the foil window impinge on the CR-39, as shown in Fig. 1(c). Each red point in the image represents the bombardment of one proton, which is visualized with software postprocessing. The screen size (80×110 μm^2) is set to be same as that in Fig. 1(b). The average number of protons per bunch irradiating the cells is measured to be $2.5 \pm 0.5 \times 10^4$ with the energy spread from 0.8 to 2.4 MeV.

The proton dose is estimated from the measured proton number and energy spectrum per bunch using a Monte Carlo simulation in the TRIM code.[11] We calculate the energy loss of the protons in a three-dimensional (3D) target consisting of the layers of 12.5-μm-thick polyimide and 5-μm-thick liquid water, which is assumed to be equivalent to the layer of the cancer cells planted on the polyimide-foil window.

Figure 2(b) shows the calculated z dependent proton energy loss distribution $d\mathcal{E}/dz$ (to the target electrons) for several beam energies in the range of $\mathcal{E}=0.8-2.5$ MeV. Here, the depth of $z=0$ μm represents the entrance (upstream) surface of the polyimide window. We note that protons with energies of $\mathcal{E}=0.8$ and 2.5 MeV leave the bottom of the polyimide window with energies of 0.16 and 2.17 MeV, respectively, and encounter the cancer cells. For $\mathcal{E} \geq 1.5$ MeV, the electronic energy loss $d\mathcal{E}/dz$ is about 20 keV/μm in the cell layer ($12.5 \leq z \leq 17.5$ μm). At lower incident energy ($\mathcal{E}=0.8$ MeV), protons have the highest $d\mathcal{E}/dz$ value and are stopped in the cell layer. The $d\mathcal{E}/dz$ used in this work is equivalent to the linear energy transfer (LET). As illustrated in Fig. 2(a), more than 85% of the protons have energy above the 1 MeV level. Belli *et al.*[12] has reported that RBE exhibits a strong dependency on the LET of the primary ions: for protons, the RBE takes a maximum with the LET range of 20–30 keV/μm. Hence, in the present experiment, the cancer cells are irradiated predominantly with protons of energy near the RBE maximum.

The energy E_d deposited on the cell layer is determined by integrating the stopping power according to: $E_d(\mathcal{E})$ $=\int_{z_1}^{z_2}(d\mathcal{E}/dz)dz$. Here, $d\mathcal{E}/dz$ is in units of keV/μm for each incident beam energy ε and integrated over the cell layer
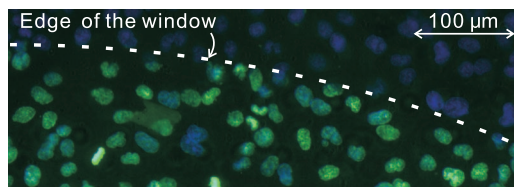
FIG. 3. (Color) γ-H2AX focus formation induced by irradiation of laser-accelerated protons with 20 Gy. γ-H2AX and nucleus are stained with anti-γ-H2AX antibody (green) and DAPI (blue).

from $z_1 = 12.5$ μm to $z_2 = 17.5$ μm. Therefore absorbed dose D of a single proton bunch is estimated (in Gy units) by the following relation: $D = \int d\mathcal{E}[E_d(\mathcal{E}) \cdot N(\mathcal{E})]/[(z_2 - z_1) \cdot Q] \cdot 1.602 \times 10^{-7}$, where $N(\mathcal{E})$ is the proton fluence distribution at the capsule entrance {in units of mm^{-2} MeV^{-1} [see the right vertical axis of Fig. 2(a)]} and Q is the mass density of liquid water in g/cm^3. We determine the absorbed dose of protons to be $D \simeq 0.1 \pm 0.02$ in a single laser shot.

DNA DSB induced on A549 are investigated by using phosphorylated histone H2AX immunostaining method. It has been recognized[13] that the H2AX phosphorylation along the DNA strand corresponds only with the site of DSB. Therefore, the remark of phosphorylated H2AX in the nuclei (termed as γ-H2AX focus formation) can be used as a criterion for DNA DSB. Figure 3 illustrates the results of γ-H2AX immunofluorescence staining obtained for the proton irradiation with 20 Gy. The proton dose was accumulated with 200 laser shots at a repetition rate of 1 Hz. Here, nuclei and γ-H2AX foci are stained with blue and green, respectively. One can easily find that γ-H2AX focus formation is detected in the nuclei. Moreover, the region of γ-H2AX positive exhibits a clear boundary along the edge of the capsule window; indicating that γ-H2AX foci are generated only in the nuclei that were irradiated with the protons. We note that we have confirmed that γ-H2AX foci are generated independently of the culture condition (see supplemental materials).[14] Therefore, we conclude that DNA DSB were induced by the irradiation of the laser-accelerated protons.

In what follows we discuss the inherent potential of laser-accelerated protons. In this study $\sim 2.5 \times 10^4$ laser-driven protons irradiate a 1 mm^2 cell layer within a time interval of only 15 ns. We then estimate the proton flux to be $\sim 10^3$ mm^{-2} ns^{-1}. On the other hand, in a typical operation of IBT,[14] tumors are irradiated with the beam with flux of $\sim 10^{-4}$ mm^{-2} ns^{-1} and a proton bunch duration of 0.4 s. Within 15 ns the present laser-driven source delivers protons with a proton number comparable to that delivered with 0.4 s duration pulse in the IBT operation. The dynamics differ by seven orders of magnitude for these cases. In radiation chemistry, it is recognized[15] that the chemical transformation induced by the energetic ions continues over the time scale of 10^{-10} to 10^{-6} s, which spans the duration of our laser-driven source of 10^{-8} s. Therefore, the laser-driven proton bunches can be a potential excitation source for the time-resolved measurements of chemical reactions including the formation and dissipation processes of hydroxyl (OH) radicals[16] in-

duced by the ion irradiation. A study such as ion-pulse radiolysis enabled by such short irradiation durations can explore the relationship between the fundamental chemical reactions of radiation effects and the ensuing biological processes.

In conclusion, we have shown that DNA DSB are generated in human cancer cells irradiated with the high-current and short-bunch ion beams driven by a laser system. It is interesting to note that about 8 proton particles, in average, were delivered to the single cancer cell nucleus in duration of the single beam bunch of 15 ns. The laser-driven *table-top* ion-irradiation apparatus will open a new field of radiobiological science and applications.

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