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Radiation Biological Application of the laser plasma x-ray and soft x-ray laser
レーザー励起単色X線ならびに軟X線レーザーを用いた放射線生物学応用

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Laser-plasma X-ray source has the attractive features such as monochromaticity, high energy, and ultra-short pulse. Soft X-ray laser produced by laser-produced plasma especially possesses excellent spatial coherence and large amount of photon number. When the laser-plasma X-ray source is applied to the biomedical investigation, it is expected that the novel phenomenon and the dynamics of biological molecules will be observed. For the first step of the biological application of the laser-plasma X-ray source, we have developed the cell irradiation system using laser-plasma X-ray source and have assessed whether DNA damage induced by the laser-plasma X-ray and soft X-ray laser is same as that of conventional X-ray source. Here, we report the recent results of the *in vitro* cell irradiation experiment.

1. Introduction

Recently, intense and ultra-short pulse X-ray from laser-produced plasma has opened a new horizon of allocations including biomedical and oncological researches. The pulse width of the laser plasma X-ray is several tens pico (10^{-12}) second and the large amount of X-ray photons are emitted within the single pulse. Moreover, the monochromaticity is superior to the conventional X-ray and therefore the X-ray can be effectively focused with X-ray optics. In addition, the spatial coherence of the X-ray laser is superior to that of conventional X-ray source. When the X-ray laser is applied to biomedical research, we can largely contribute to the progression of biomedicine.

The purpose of this research is to apply the laser plasma X-ray and soft X-ray laser to the biomedical research.

2. Radiation biological effect of the laser-plasma X-ray

First, we have revealed whether radiation biological effect of laser-plasma X-ray is same as that of conventional X-ray. To generate the laser-plasma X-ray, high intensity Ti;sapphire laser was used. The laser intensity, pulse width and repetition were 3.0×10^{17} W/cm^2, 70 fs, and 10 Hz, respectively. The laser spot size was 30 μm on the target surface. In this system, the copper (Cu) slab was used for the target and therefore the 8 KeV X-ray, which is Cu Kα-line, was emitted from the target. The X-ray was focused with the poly-capillary X-ray lens into the culture cells. The absorbed dose of the laser-plasma X-ray was measured with Gafchromic EBT film.

In all experiments, the human lung adenocarcinoma cell line A549 was used. The silicon nitride (SiN) membrane was used for the cell culture substrate. A certain number of A549 were seeded onto the SiN membrane and incubated with 10 % fetal bovine serum (FBS) containing Dulbecco's modified Eagle's medium (DMEM). The cell culture dish we used in the irradiation experiment has the 3 mm diameter hole at the center of bottom side. Immediately before the irradiation, the SiN membrane in which the cells were adhered on the surface was attached to the hole of cell culture dish. The irradiation site was positioned by the upright microscope and automatic stage that installed on the irradiation system. After the positioning, the cells were irradiated with laser-plasma X-ray at room temperature.

To measure the absorbed dose of the laser-plasma X-ray, the changes in color density of the Gafchromic EBT film was analyzed. After the irradiation, the Gafchromic EBT film was scanned with the flatbed commercial scanner and the density profile was acquired. The color density was calibrated by the high energy X-ray generated with the 4 MV medical LINAC. As the results, the absorbed dose was 0.048 Gy/min. The X-ray spot size was ranging from 785 to 954 and 796 to 976 μm along the horizontal and vertical direction, respectively.

The results of the film dosimetry indicated that the 40 to 50 min. of the irradiation time was

needed to treat the cells with 2 Gy. To confirm whether the DNA double strand break (DSB) is induced by the laser-plasma X-ray, we irradiated the A549 with 2 Gy of laser-plasma X-ray and then performed the immunofluorescence staining with anti-γ-H2AX antibody. As the result, the region of γ-H2AX positive cells was clearly identified as the circular region. The diameter of this region is 922 μm along the horizontal direction and the value approximately corresponded with the X-ray spot size measured by Gafchromic EBT film dosimetry. To investigate whether the radiation biological effect of laser-plasma X-ray was same as that of the conventional X-ray, we performed the immunofluorescence staining with anti-γ-H2AX and anti-p-ATM antibody and quantified the number of γ-H2AX and p-ATM focus in the nucleus. As the results, the number of γ-H2AX focus induced by the laser-plasma X-ray were same as that induced by high energy X-ray. Likewise, the number of the p-ATM focus showed same aspects as the number of the γ-H2AX focus. These results demonstrated that the radiation biological effect of laser-plasma X-ray was same as that of conventional X-ray such as high energy X-ray generated from medical LINAC. It was confirmed that the laser-plasma X-ray could be used for the biomedical study [1, 2].

3. Soft X-ray laser-induced DNA double strand breaks

We have applied the soft X-ray laser to the radiation biological study and investigated whether the DSB was induced by the soft X-ray laser irradiation. The soft X-ray laser used in this study was generated by double-target method developing at JAEA. Briefly, Two silver (Ag) slab target were irradiated with Nd:glass laser to generate the highly-charged plasma (Nickel like Ag-ions) and 90 eV soft X-ray laser are emitted from the plasma generated in each target. The output energy, pulse width, photon number per laser shot and X-ray spot size at the surface of incidence were approximately 300 nJ, 7 ps, 3.0×10^{10} photons and 90 μm in diameter, respectively. The soft X-ray laser was vertically reflected with molybdenum/silicon (Mo/Si) mirror to be incident to the cell culture substrate.

The energy of soft X-ray laser used in this study is 90 eV and therefore the X-ray photons are easily absorbed by the air and water. This problem is most critical for the radiobiological study because the irradiation experiment must be performed at the air to keep cell living. For this reason, we developed the custom-made irradiation dish to irradiate cells with soft X-ray laser. The dish is made of Aluminum and a hole is opened at the center of the irradiation dish. By attaching the SiN membrane to a hole, this dish can be used for the vacuum window of the target chamber. In addition, the inside of the dish can be filled with the culture medium and therefore the cells can be cultured on the upper side of SiN membrane. The cells can be irradiated with the soft X-ray laser regardless of the X-ray attenuation by any air and water by using the irradiation dish we designed.

To investigate whether the DSB is induced by the soft X-ray laser, we perform the immunofluorescence staining with anti-p-ATM and p-DNA-PKcs antibody. As the result, p-ATM and p-DNA-PKcs were identified in the nucleus even after the single shot of soft X-ray laser although the photons of the soft X-ray laser is almost absorbed by the cellular membrane and cytoplasm and therefore is not reached nucleus. These data suggest that the DNA damage response is induced by the the large amount of energy transfer into the cellular membrane and cytoplasm. The reproducibility of this result must be testified by the further irradiation experiments as soon as possible.

4. Conclusion

In this research, We conformed that the effect of laser plasma X-ray on the DSB were same as that of conventional X-ray and that 90 eV soft X-ray laser could induce the DSB although the x-ray energy was not enough to penetrate the cellular membrane, cytoplasm, and nucleus.

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