Ion-microbeam irradiation to damage DNA in RPE cell nucleus with tapered glass capillary optics at Pelletron facility

T. Ikeda,[*1] M. Sakai,[*2,*1] K. Takemoto,[*2,*1] A. Shibata,[*3] M. Izumi,[*1] and M. Uesaka[*2]

Cell irradiation experiments with ion microbeams have started at a new beamline of the RIKEN Pelletron accelerator facility in the Nishina R&D Building. The purpose of the experiments is to investigate the dynamics of the accumulation of restoration proteins after DNA double-strand breaks (DSB). The microbeam irradiation allows artificial DNA damage, which defines the time zero for the accumulation of repair proteins. To create DSB efficiently, ions with high linear energy transfer (LET) should be used. Figure 1 shows the LET in water for H and He ions with an energy E below 5 MeV. The H and He ions have maximum LETs of 80 and 240 keV/μm, respectively, just before stopping. If we use He ions with an energy of 4.5 MeV, an LET of more than 100 keV/μm is available through an ion range of 27 μm. The LET value is large enough to create DSB.

The irradiation was performed at the BL-W30 beamline employing a single tapered glass capillary optics at the beam port, where the microbeam was emitted in the horizontal direction. A He-ion beam was accelerated to 4.5 MeV with a tandem accelerator (Pelletron 5SDH-2, 1.7 MV max.), and it entered a glass capillary having an outlet diameter and end window thickness of 11.4 μm and 8.6 μm, respectively. The details of the capillary microbeam at an old beamline of our facility are reported elsewhere.[1,2] The target was nuclei of retinal pigment epithelial (RPE) cells cultivated in a liquid medium. The cells were fully confluent in petri dishes, the glass bottoms of which were marked to find irradiated cells for the fluorescence observation after the irradiation. As shown in Fig. 2(a), the petri dish was positioned at an angle of approximately 45° to the capillary axis. To maintain the cells in the dish filled with the medium, it was dripped onto the bottom of the dish at a speed controlled by a peristaltic pump. The microbeam intensity was set to approximately 15 ions/s, which was confirmed before and after the irradiation. The irradiation distance between the capillary outlet and the cells was set to several micrometers. The distance was always monitored by a microscope above the irradiation point. Each dish was moved for 3–8 min to irradiate the cells on a line across the dish. The total number of dishes was four. After the irradiation, 30 min was spent for the repair process based on an enzymatic reaction. Subsequently, the cells were fixed with 3% paraformaldehyde (PFA)-2% sucrose in phosphate-buffered saline (PBS), followed by washing with PBS. Next, the dishes were filled with PBS for delivery to Gunma University for observation by an optical microscope with three-dimensional structured illumination, 3D-SIM, microscopy (DeltaVision OMX version 4, GE Healthcare).

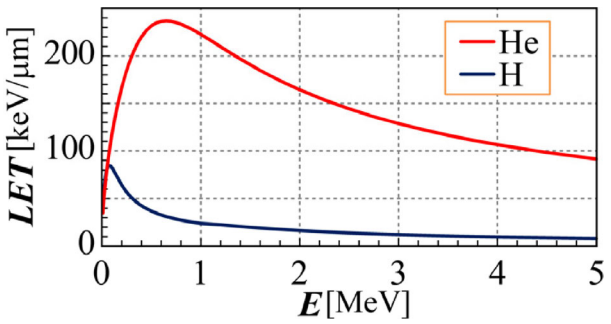

Fig. 1. Comparison of the LETs of H and He ions in water with an energy between 0 and 5 MeV.

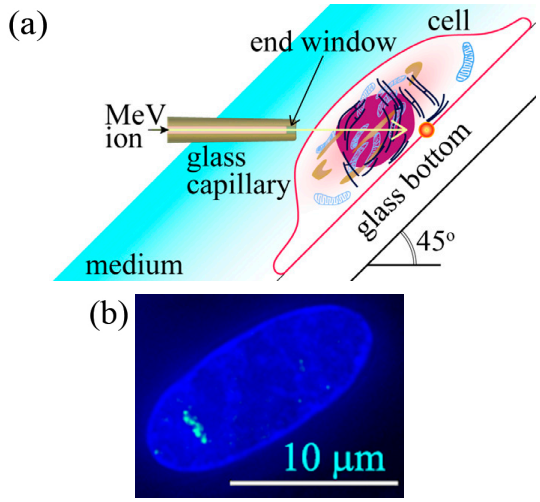

Fig. 2. (a) Cell irradiation with a glass capillary. The angle was set to approximately 45° with respect to the capillary axis. (b) Irradiated RPE cell nucleus with DSB points.

Figure 2(b) shows a bottom view of one of the irradiated cells. The blue area corresponds to the cell nucleus labeled by DAPI. The bright blue points are DSB positions where phosphorylated histone H2AX was labeled with Alexa Fluor® 488 by the immunofluorescence method. The DSB points are clearly aligned on a line with extremely low background fluorescence. The line is expected to be a He ion track stopping at or penetrating the nucleus. The photo is one of the z-sliced photos with a slice step Δz of 0.25 μm.

The brightness information of each pixel in the images was stored as a 3D map. Using the map, the directions of all ion tracks and the density distributions of DSB and other restoration proteins were extracted. The time evolution of repair of DSB as a function of the distance from the ion track will be obtained in the next experiment by changing the time between irradiation and fixing.

*1 RIKEN Nishina Center
*2 The Graduate School of Engineering, Univ. of Tokyo
*3 Gunma University Initiative for Advanced Research (GIAR), Gunma Univ.

References

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