Detection of DNA Damage by Space Radiation in Human Fibroblasts Flown on the International Space Station

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Introduction

Space radiation consists of energetic charged particles of varying charges and energies. Exposure of astronauts to space radiation on future long duration missions to Mars, or missions back to the Moon, is expected to result in deleterious consequences such as cancer and comprised central nervous system (CNS) functions. Space radiation can also cause mutation in microorganisms, and potentially influence the evolution of life in space. Measurement of the space radiation environment has been conducted since the very beginning of the space program.

Compared to the quantification of the space radiation environment using physical detectors, reports on the direct measurement of biological consequences of space radiation exposure have been limited, due primarily to the low dose and low dose rate nature of the environment. Most of the biological assays fail to detect the radiation effects at acute doses that are lower than 5 cSv.

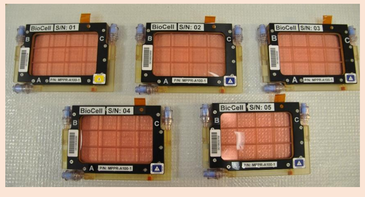
In a recent study, we flew cultured confluent human fibroblasts in mostly G1 phase of the cell cycle to the International Space Station (ISS). The cells were fixed in space after arriving on the ISS for 3 and 14 days, respectively. The fixed cells were later returned to the ground and subsequently stained with the γ-H2AX antibody that are commonly used as a marker for DNA damage, particularly DNA double strand breaks, induced by both low- and high-LET radiation. In our present study, the γ-H2AX foci were captured with a laser confocal microscope. To confirm that some large track-like foci were from space radiation exposure, we also exposed, on the ground, the same type of cells to both low- and high-LET protons, and high-LET Fe ions. In addition, we exposed the cells to low dose rate γ rays, in order to rule out the possibility that the large track-like foci can be induced by chronic low-LET radiation.

Materials and Methods

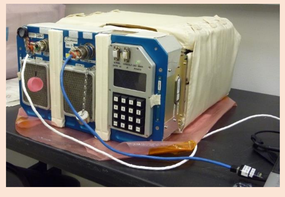
Normal human fibroblasts AG1522 were grown to confluency in alpha-MEM with 10% FBS and antibiotics in humidified tissue culture chamber at 37˚C with 5% CO_2 and flown to the International Space Station (ISS) using BioCells cell culture chambers manufactured by BioServe Space Technologies, University of Colorado. The cells were let stay on ISS for 3 or 14 days before fixed with 2% formaldehyde and stored at 4˚C. Control experiments were done on the earth following the same experimental protocol. Upon returning to the earth, the fixed cells were stained γ-H2AX antibody and the stained slides were scanned with a Leica laser confocal microscope with a 60X objective lens at a step of 0.3 μm. Images were analyzed with Imaris version 7.6.3 (Bitplane Technologies, Zurich Switzerland).

For ground experiment with low dose rate γ rays, confluent AG1522 cells in chamber slides were exposed to a low activity Cs-137 γ source at NASA Johnson Space Center, Houston, Texas for 14 days continuously in an incubator that was maintained at 37˚C during the exposure period. The dose rate at the sample was 0.045 cGy/hr and the accumulate dose over the period of 2 weeks was 16.3 cGy. At the end of exposure period, the cells were washed with PBS and fixed with 2% paraformaldehyde for 15 min at room temperature. The samples were analyzed as above.

For ground experiment with charged particles, AG1522 cells cultured in chamber slide were maintained in the same culture solution as in the flight experiment in an incubator at 37 0C. Cells at confluence were exposed to 150 MeV protons at entrance and near the Bragg peak, as well as 600 MeV/u Fe ions, at the NASA Space Radiation Laboratory located in Brookhaven National Laboratory, New York.



(a)



(b)

Figure 1. Flight hardware used in the study onboard the ISS. (a) BioCells cell culture chamber used for the experiments. Cells were grown on only the bottom surface. (b) The cells were kept in a CGBA (BioServe Space Technologies) incubator at 37˚C.

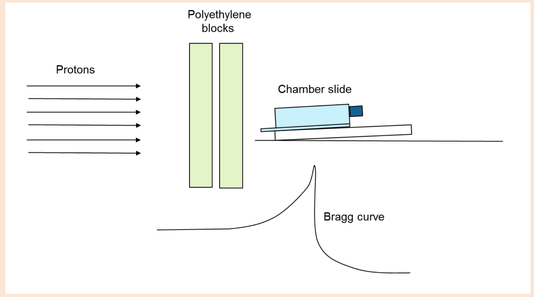


Figure 2. Human fibroblast cells exposed to energetic particles. Polyethylene blocks were place in front of the chamber slides to achieve high-LET protons at the location of the Bragg peak in the middle of the slide. For Fe ions and low-LET protons, no polyethylene blocks were used.

Results

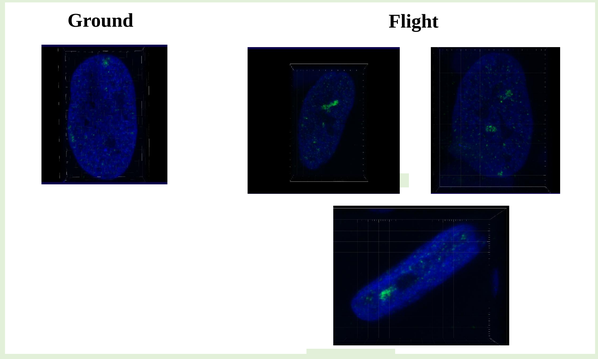


Figure 3. Images of γ-H2AX foci in human fibroblast cells that had been flown in space for 14 days and on the ground.

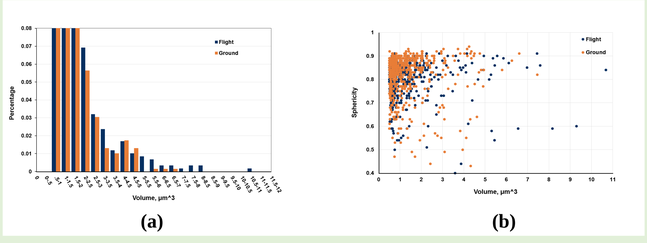


Figure 4. (a) Volume distribution of the γ-H2AX foci in flown and ground cells fixed on Day 14. Several large size foci were observed in the flown cells. (4) Sphericity of foci in cells fixed on day 14 between ground and space.

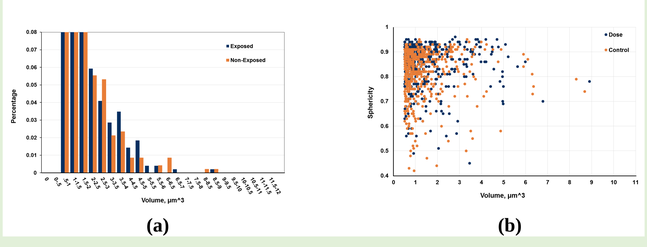


Figure 5. (a) Volume distribution of the γ-H2AX foci in the cells exposed to low dose rate γ rays for 14 days on the ground in comparison to the unexposed controls. (b) Sphericity of foci in the cells exposed to low dose rate γ rays on the ground in comparison to the unexposed cells. .

Results (Continued)

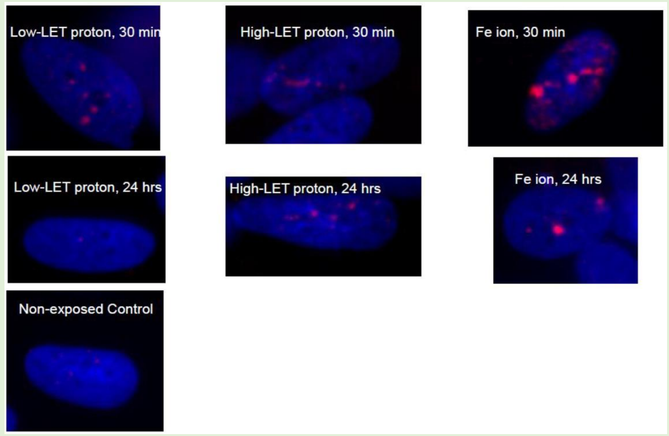


Figure 6. γ-H2AX tracks found in flight samples were most likely induced by high-LET protons or high-LET heavy ions.

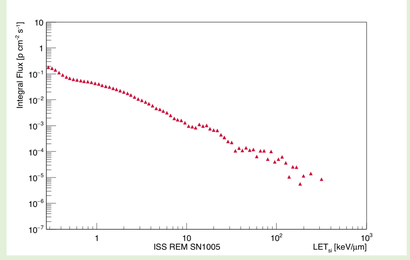


Figure 7. Integral LET spectrum measured on the orbit of ISS around the time of the present spaceflight experiment.

Conclusions

Measurement of biological effects of space radiation is challenging due to the low dose and low dose rate nature of the radiation environment, and due to the difficulty in distinguishing the radiation effects from microgravity and other space environmental factors. Human fibroblasts were exposed to low- and high-LET protons, and high-LET Fe ions on the ground. Our and others' results suggest that DNA damage induced by high energy, heavy ions in space radiation may not be repaired in weeks after initial incident. Cells flown to ISS showed similar number of foci per nucleus and similar average foci size for γ-H2AX foci. However, there were a few large γ-H2AX foci in cells flown to ISS but not in ground control samples. Also in a separate ground experiment, cells exposed to chronic gamma rays showed similar foci size distribution in comparison to the non-exposed controls. Our results suggest that in G1 human fibroblasts under the normal culture condition, only a small fraction of large size foci can be attributed to high-LET radiation in space.

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