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Radiation Fields, Effects and Risks in Human Space Missions (5)

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INVESTIGATION OF CELLULAR RESPONSES TO SPACE-RADIATION SIMULATED WITH PROTON BEAM APPLICATION IN DENTAL PULP STEM CELLS

Abstract

Years of spaceflight and subsequent in vitro research have demonstrated that cells can respond structurally and functionally differently to environmental changes in space. Proton radiation represents the biggest contribution to the dose for organic cells in a space habitat. To better predict the effects of space proton radiation on Dental Pulp Stem Cells (DPSCs), tests were performed at METU IVMER DBL radiation test facility in this study. The aim of this work is to reveal, for the first time, the cellular responses of DPSCs to proton radiation. DPSCs cultured in alpha-MEM/20 percentage of FBS at 37C. The cell surface markers of the P3 cells were examined by flow cytometry (NovoCyte) for CD105, CD90, and CD73 markers. Osteogenic capacity of DPSCs was evaluated by QuantiChrom Calcium Assay Kit and Stempro Osteogenesis Differentiation Kit at day 14. Radiation dose calculations for DPSC samples with 30 MeV protons were performed using Monte Carlo simulations with SRIM software. LET values in various materials and packaging thicknesses were calculated. A sample bag that contains the DPSCs was produced from thin layers of polypropylene, a material commonly used in medical infusion bags, with a total thickness of approximately 0.5 mm. A radiation-tolerant temperature-control unit was developed to main- tain cell temperature at 37C during transportation and irradiation processes. This unit is controlled by a PID algorithm on an ESP-32 micro-controller and equipped with a battery pack to last for 16 hours. Live temperature data can be monitored via a Grafana interface. After radiation, the cell surface markers of the P3 cells were examined for cancer markers; CD44 and CD133. The expression of genes specific to stress condition was quantitatively determined by RT-qPCR. Expressions of SOD1, XBP1, p21 and reference gene GAPDH were measured comparatively using Light Cycler 480 II PCR device (n=3). Western Blot was performed to assess the effect of radiation on DPSCs (n=3). For this purpose, total protein extraction was performed by RIPA lysis buffer with protease inhibitor cocktail. Pro-apoptotic Bax, anti-apoptotic Bcl-2 and GAPDH were used for protein quantification. According to our results, DPSCs displayed stress responses determined by gene expression levels in antioxidant system (SOD1) and DNA damage and double strand break repair activity (XBP1). p21 levels were evaluated to determine the fate of cells for DNA repair process and radio resistance. Our results suggest that radiation have a negative impact on DPSCs survival (Bax/Bcl-2) but also impact on differentiation.