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FLASH irradiation of in-vitro samples with a modified X-Ray tube

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Purpose
FLASH radiotherapy is a promising technique in radiotherapy, where ultra-high dose rates (>40 Gy/s) have been shown in in-vitro and animal studies to have a protective effect on healthy tissues, while maintaining the same efficacy in treating tumors as conventional radiotherapy. We performed a survival and viability assay with an X-ray beam at FLASH and conventional dose rates, using healthy lung fibroblasts and lung cancer cell lines to study potential biological mechanisms that could be at the root of the observed differential FLASH effect.

Materials and methods
Healthy lung fibroblasts (CCD19) and lung adenocarcinoma cells (A549) were cultured in regular DMEM supplemented with 10% FBS and penicillin/streptomycin under standard culturing conditions. Two different seeding conditions were irradiated. On one hand, 24h before the irradiation the A549 cell line was seeded in MW96 plates. On the other hand, 1·10^6 tumor cells and 4.5·10^5 healthy cells were collected and pellet in eppendorfs vials.
Both seeding conditions were irradiated with 0-30 Gy using a modified X-ray tube at FLASH (150 kVp and 500 mA) dose rates (34 Gy/s-88 Gy/s) and conventional (150 kVp and 10 mA) dose rates (0.68 Gy/s-1.72 Gy/s). Doses and dose rates were determined via radiochromic films (RCF) dosimetry.
In vitro clonogenic study was performed on A549 and CCD19 cells by seeding increasing concentrations with the dose of irradiated cells in MW6 plates. Ten days later, cells were fixed, stained with crystal violet and number of colonies quantified with a self-developed Matlab script, while the viability assay done with the healthy cells was manually quantified. Both data were analyzed with the linear quadratic model.

Results
Dosimetric equivalence FLASH vs. CONV was confirmed as long as the delivered charge remained constant. Preliminary analysis of the results (still ongoing) suggests no differential biological effects related to the dose rate that is statistically significant. Clonogenic assays for the A549 cells showed no differential dose-rate effect in the biological response, as well as the viability assays conducted with the CCD19 cell line.

Discussion and conclusions
On one hand, via radiochromic film dosimetry we were able to determine the dose rates used in the experiment and the exact dose irradiated in each shot to each eppendorf vial or MW96 plate well.
On the other hand, equivalent biological results were obtained in an in vitro study at normoxic conditions between X-ray irradiations at conventional and FLASH dose rates for both healthy fibroblasts and lung cancer cells.

Abstract

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