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Effectiveness of laser accelerated ultra high dose rate protons in DNA DSB damage induction under hypoxic conditions

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Particle therapy has been regarded as an effective modality for treating tumors due to higher RBE than photons and precise dose delivery to the deep seated tumors near the critical organs offering significantly sparing of the normal tissues¹. High LET ions have been reported to kill radioresistant and hypoxic tumor cells^{2,3}. The large footprint, installation and operational costs of the current particle therapy facilities pose a challenge towards the widespread accessibility of particle therapy. High-power lasers have successfully demonstrated the generation of ion beams⁴⁻⁶, which have been proposed as a route towards reducing the overall footprint and costs of future hadrontherapy facilities⁷.

In view of this potential future application a detailed radiobiology of the interaction of laser accelerated ions beams with biological systems is highly warranted. Radiation induced reactive oxygen species (ROS) lead to cellular damage through the induction of an array of lesions including DNA base damage, single strand breaks and double strand breaks and induce apoptosis and necrotic cell death⁸⁻¹⁰. Under hypoxia, the proliferation rate of tumor cells beats the vasculature formation rate leading to the development of oxygen deficient zones within the tumor¹¹. Such hypoxic zones leads to reduced radiotherapy response owing to a decrease in ROS that are required to produce lethal DNA damage¹²⁻¹⁴.

In this paper we aim to study the effectiveness of laser-accelerated protons in the induction of DNA DSB damage in hypoxic human cells through a comparison to 225 kVp X-rays and cyclotron accelerated protons.

Cell Culture: Normal human skin fibroblast cells (AG01522) were cultured in Minimum Essential Medium (α -modified, Sigma Aldrich) supplemented with 20% FBS and antibiotics and incubated in 5% CO₂ with 95% humidity at 37°C in a CO₂ incubator. A day before irradiations, the cells were seeded on custom made steel dishes and incubated overnight.

Hypoxia Chamber design and hypoxia induction: The hypoxia chamber is box shaped and is provided with two metal gas ports, one inlet and one outlet for connecting the hypoxic gas. The cells were grown on custom-made steel dishes consisting of stretched Mylar to allow cells to grow in monolayers. A day after seeding, these dishes were attached inside the hypoxia chamber and were gassed for 4 hours for hypoxia induction while maintaining the optimal incubation temperature and humidity.

Irradiations: Irradiations with X-Rays were carried out using the 225 kVp X-Ray generator at our institute and Laser accelerated proton irradiations were carried out employing the petawatt arm of the VULCAN laser facility at the Rutherford Appleton Laboratory, Oxford UK. After irradiation the cells were fixed using 4% paraformaldehyde solution at stipulated time intervals for studying the biomarkers of hypoxia and DNA DSB damage.

Hypoxia and DNA DSB damage detection: After fixation the samples were washed with PBS (Phosphate Buffered Saline) and later permeabilized with 0.5 % Triton X-100. Subsequently the cells were blocked in 2 ml of blocking buffer containing 10% goat serum and 0.25 % Triton X-100 and incubated at 37 °C for 2 hours. The samples were then stained for both 53BP1, a biomarker of DNA DSB damage, and Hypoxia Inducible Factor-1 α (HIF-1 α). The cells were later probed with secondary antibodies conjugated to Alexa Fluor 488 and Alexa Fluor 594 specific to 53BP1 and HIF-1 α . The samples were then washed and mounted on coverslips using an anti-fade reagent containing DAPI.

Results, Discussion and Conclusion:

We measured the hypoxia induction using HIF-1 α in cells and the physical oxygen sensing probe Rapidox meter, capable of measuring the real-time oxygen concentration, as shown in figure-1.

The figure shows the comparison of the oxygen concentration over time for three different chambers. A

consistent anoxic level (<0.5 % Oxygen) was maintained even after 40 minutes of disconnecting the chambers from the gas supply showing the pressure retention ability of the chambers

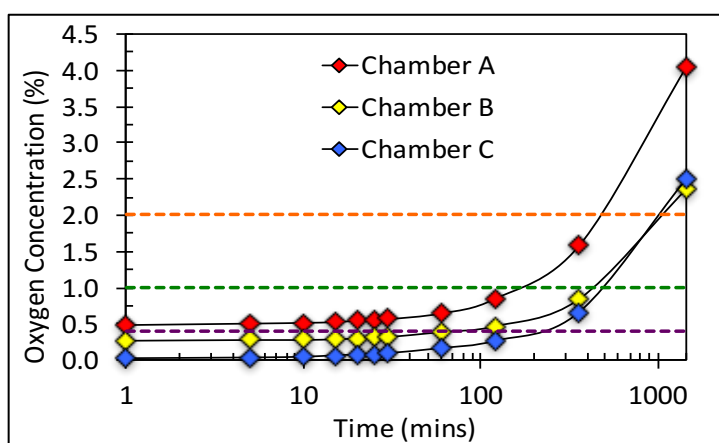


Figure-1. Oxygen concentration kinetics for the 3 different hypoxia chambers measured using Oxygen sensing probe Rapidox Sensotec meter.

contributing towards their portability. The portability is an important aspect as the sample preparation facility is typically away from the irradiation beam line.

Biological validation of hypoxia is shown in figure-2, in the normoxic cells the HIF-1 α (shown in red) expression was very low compared to the hypoxic cells where HIF-1 α clearly localized to the nucleus after four hours of gassing with hypoxic gas. Radiation induced DNA DSB plays an important role in the cell death of tumor cells and

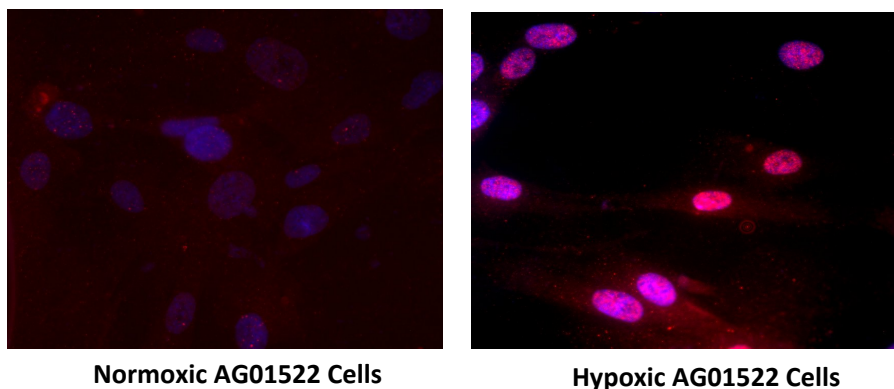


Figure-2. HIF-1 α expression in AG01522 cells stained using primary antibody against HIF-1 α tagged with secondary antibody conjugated to Alexa fluor 568.

has been the basis for development of a number of cancer treatment modalities¹⁵⁻¹⁷. 53BP1 protein phosphorylation in response to DNA DSB has been used as a well-reported surrogate marker of DNA DSB damage¹⁸. We also used 53BP1 foci for the quantification of the laser-accelerated protons induced DNA DSB damage as shown in figure-3 where the 53BP1 foci are shown in green and yellow (when the foci are overlapping the HIF-1). Our results indicate that ultra high dose rate protons induce similar 53BP1 foci as induced by any low LET radiation such as X-rays and protons. At 30 minutes and 24 hrs a close similarity is seen between 15 MeV laser accelerated protons and 15 MeV cyclotron generated protons. For measuring the effectiveness of laser-accelerated

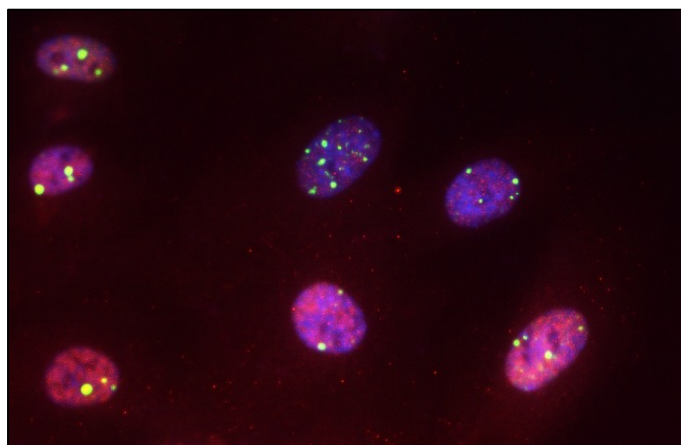


Figure-3. 53BP1 foci formation (green) in hypoxic AG01522 cells 6 hours after exposure to 1 Gy 15 MeV laser accelerated protons.

protons under hypoxia we compared the foci numbers at 30 minutes under normoxic to hypoxic irradiations and obtained an oxygen enhancement ratio (OER) as 1.84 ± 0.2 . At 24 hrs. the OER was 1.08 ± 0.38 showing the effective cell killing by laser-accelerated particles irrespective of

the oxygenation status of the cells. Through our paper we have shown the first measurement of DNA DSB damage induced by laser accelerated protons under hypoxia. While the initial DNA DSB damage induced by laser-accelerated protons appears similar to any low LET particle the residual damage is slightly higher than for X-rays under hypoxia. Further experiments involving more varied cell lines and endpoints are warranted.

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