Experimental Study of Radiolytic Oxygen Depletion from X-ray Irradiation in Water and Liquid Samples

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Abstract

The mechanism underlying the reduction of toxicity in normal tissue caused by ultra-high dose rate irradiation (FLASH) is being investigated but still unknown. Oxygen depletion or the interaction of radiation-induced radicals with dissolved oxygen during irradiation may explain the FLASH effect. In this study, various liquid samples, including full oxygenated water, deoxygenated water, phosphate-buffered saline (PBS) and Ham's F12 cell culture medium were irradiated with conventional dose rates to investigate experimental oxygen depletion in samples. The radiation-induced radical production and reactions were examined as a reference, before studying the oxygen depletion with FLASH irradiation. Samples were exposed to 50 Gy of X-ray radiation at a dose rate of 4.5–10.4 Gy/min. X-ray source dosimetry was performed using a Semiflex ionization chamber. A chemical optical sensor was used to measure the amount of oxygen, and the results were recorded online. The oxygen depletion was highest in the first irradiation step. The result showed that the depletion of oxygen in Ham's F12 cell culture medium was highest with the value of 0.42 µM/Gy. Deoxygenated water and phosphate-buffered saline (PBS) had the lowest oxygen depletion with the value of 0.26 µM/Gy. For the irradiation in the second step, the results showed a similar trend of oxygen depletion that was lower than the first step and lowest depletion occurred in the third step of irradiation. We conclude that radical production and reactions, which compete with oxygen depletion, cause the declining behavior of oxygen depletion. Additionally, solutions containing organic compounds showed a greater depletion of oxygen.

Keywords**:** *X-ray irradiation, FLASH irradiation, FLASH effect, Oxygen depletion*

1. Introduction

One essential part of the cancer treatments available today is radiotherapy. Although it is well established for clinical treatment, irradiation at conventional dose rates (CONV) (~ 2 Gy/min) takes time to deliver the treatment. Moreover, the irradiation process damages not only tumor cells but also normal tissues.

In fact, the irradiation experiment with ultrahigh dose rates has been reported since the 1950s (Dewey & Boag, 1959; Town, 1967; Hornsey & Bewley, 1971), and the term of "FLASH" irradiation (in excess of 40 Gy/s) was applied in 2014 and has gained much focus over the last few years. The *in vivo* experiment using FLASH irradiation was performed in a mice tissue. The result showed radio-protection in the normal tissues while preserving cytotoxicity in tumor tissues. This normal tissue sparing effect is referred to as the FLASH effect (Favaudon et al., 2014). Currently, there are different FLASH sources consisting of photons, electrons protons and carbon ions (Montay-Gruel et al., 2018; Liljedahl et al., 2022; Sørensen et al., 2022; Tinganelli et al., 2022). Although FLASH irradiation has been demonstrated, both *in vivo* and *in vitro* (Vozenin et al., 2019; Adrian et al., 2020; Hageman, Che, Dahele, Slotman, & Sminia, 2022), but the mechanism behind the FLASH effect is still unclear. Recently, the first human trial showed that FLASH therapy was effective in treating cancer, but its efficacy was comparable to conventional radiation therapy (Bourhis et al., 2019; Gaide et al, 2022). Oxygen depletion is thought to be the FLASH mechanism (Adrian et al., 2020; Pratx & Kapp, 2019).

Oxygen is a powerful radiosensitizer that generates a free radical molecule that has efficiency for DNA damage (Liu et al., 2015). Several studies demonstrated that the cells in hypoxic conditions, or with

hypoxic areas, are up to three times more radioresistant than normal cells at the well-oxygenated level (Grimes & Partridge, 2015). During irradiation, ionizing radiation induced water radiolysis cause the dissociation of water molecules, which leads to the loss of dissolved oxygen (Le Caër, 2011; Boscolo, Krämer, Fuss, Durante, & Scifoni, 2020) and generates a wide range of chemical species in a short period of time. Hydrogen radicals and solvated electrons are among the major apparent products of irradiation. These radiolytic species can interact with molecular oxygen dissolved in water, then produce the perhydroxyl ($HO_2^{\bullet}$) radical and superoxide ($O_2^{\bullet-}$) resulting in a decrease in oxygen concentration. Early findings in oxygen measurements in bacteria and mammalian cells showed that oxygen depletion can lead to significant at very high dose rate sparing by having the cell survival curves behave as in a hypoxic condition during irradiation (Epp, Weiss, Djordjevic, & Santomasso, 1972; Weiss, Epp, Heslin, Ling, & Santomasso, 1974). Moreover, investigations of oxygen depletion have recently been published as part of experiments and simulations (Jansen et al., 2021; Boscolo, Scifoni, Durante, Krämer, & Fuss, 2021).

In this study, we investigated the oxygen depletion in irradiated water and liquid samples consisting of full oxygenated water, deoxygenated water, phosphate-buffered saline (PBS) and Ham's F12 cell culture medium with CONV dose rate range. X-ray beam was used as the radiation source since it is the typical beam that is usually used for the CONV radiotherapy.

2. Objectives

1) To investigate oxygen depletion in irradiated samples such as full oxygenated water, deoxygenated water, phosphate-buffered saline (PBS) and the cell culture medium.
2) To compare oxygen depletion between the different samples.
3) To examine the radical production and reactions that occur from the x-ray irradiated samples and keep the data as a reference before doing the experiment on FLASH irradiation.

3. Materials and Methods

3.1 Instruments for oxygen measurement

For the experimental section of this study, almost all the equipment involved in this oxygen depletion measurement was from PreSens Precision Sensing GmbH (Germany). There were two types of containers used in this experiment. The first container was the main container for X-ray irradiation only. It was designed for non-vacuum conditions which could be reused and cleaned (see Figure 1(a)). The second container was the cylindrical container as shown in Figure 1(b). It was designed for both X-ray and FLASH irradiation. Each container, the sensor spot with a diameter of 5 mm (SP-Pst3-SA23-D5-OIW-US) was glued on the inner wall. This sensor spot contains luminophores for detecting the oxygen molecules dissolved in the samples (Ast, Schmälzlin, Löhmannsröben, & van Dongen, 2012). During the measurement, the oxygen concentration was monitored and collected by a compact oxygen meter system (OXY-1 SMA-trace-RS232-AO) and the PreSens.EOM STS software. The LED in this oxygen meter excites the sensor spot at a 505 nm wavelength. After that, the oxygen meter processor continued to work based on the phase signal. The changes in oxygen concentration inside the samples were measured with a time resolution of 1 s.

3.2 Container setups and samples preparation

Two types of container were used in this experiment, the main container and the cylindrical container. The main container had the benefit of being reusable. The cylindrical container was airtight and used in vacuum conditions. The main container consisted of five parts: a spot sensor, a holder, an inset, a rubber stopper and a plastic container. The spot sensor was located at the center between the plastic container and the inset. The optical fiber was connected to the plastic container via a holder piece, which was placed in the same location as the spot sensor. The inset was an area for filling samples. One side of the inset was opened to allow the sensor spot to contact the sample. Before inserting the inset inside the plastic container, we coated the exterior of the inset with a moisture-curing silicone rubber (ELASTOSIL® E43 TRANSPARENT). The rubber stopper was then used to seal the inset compartment, keeping it airtight and

preventing the diffusion of oxygen. The inset can hold a sample with a volume of approximately 1 cm^3. The total height of the main container, including the lid is 5.4 cm.

The cylindrical containers (see Figure 1(b)) were prepared for both X-rays and FLASH experiments. It consists of three cylindrical components. The top part has been tapped with a threading for mounting a screw to maintain the proper position of the container. The middle part is a one-open-end cylinder with a 5 mm inner diameter and a 5 mm depth. At the open end, it is covered with a thin piece of transparent plastic with a spot sensor placed underneath. Another side of the cylinder has a tiny hole for filling the container with the sample using a syringe. The bottom part is an optical fiber holder that allows the optical fiber to make a contact with the sensor spot.

An inset piece of the main container and three pieces of the cylindrical container were made in the GSI Helmholtz Center for Heavy Ion Research (GSI) workshop using polyetheretherketone (PEEK) material. It is resistant to radiation and chemicals. When exposed to radiation, it does not leach chemicals or oxygen.

Samples were prepared differently depending on their properties. Full oxygenated water, phosphate-buffered saline (PBS) and cell culture medium (Ham's F12) do not need any special preparation. They can be filled directly into containers. However, for the deoxygenated water, nitrogen gas was added into the full oxygenated water for 10–15 minutes to decrease the oxygen concentration from 250-320 µM to 189–235 µM.

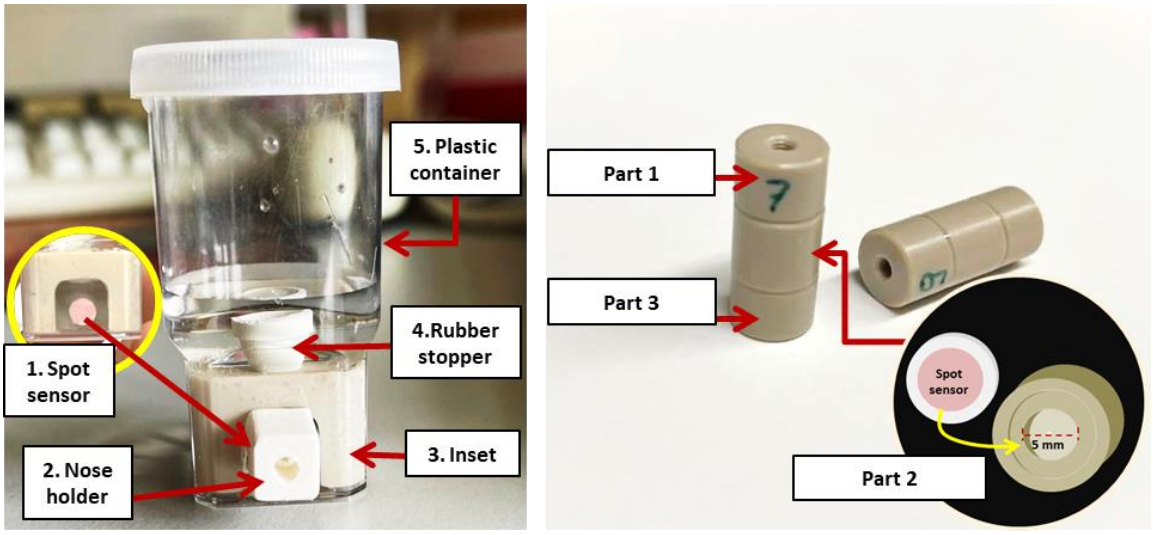

Figure 1 Apparatus of the study: (a) the main container for X-ray irradiation used to contain the water and PBS samples. (b) The cylindrical containers with an inner diameter of 5 mm and a depth of 5 mm used for FLASH experiments in vacuum conditions.

3.3 X-ray beam and dosimetry

To investigate the oxygen depletion at the conventional dose rate, the samples with the sensor spots were irradiated using a 250 kV X-ray beam at the GSI Helmholtz Centre for Heavy Ion Research, Germany at a dose rate of 4.5–4.8 Gy/min for the large container and 10.0–10.4 Gy/min for the cylindrical container. The dosimetry for the X-ray irradiation was carried out using a Semiflex ionization chamber (IC, type number TM31013, PTW, Germany).

3.4 Experimental setup

For the irradiation, both containers were fully filled with samples without air bubbles and completely closed. Containers were placed under the X-ray target and beside the Semiflex chamber. On the outside of the container wall, the optical fiber with a total length of 4 meters was placed to connect between the sensor

spot and the oxygen meter. The oxygen meter was then linked to the computer for data collection. Figure 2 shows the experimental setup for X-ray radiation. A calibration was done for the oxygen meter prior to use in the specific set-up (with respective container, cable length and connectors).

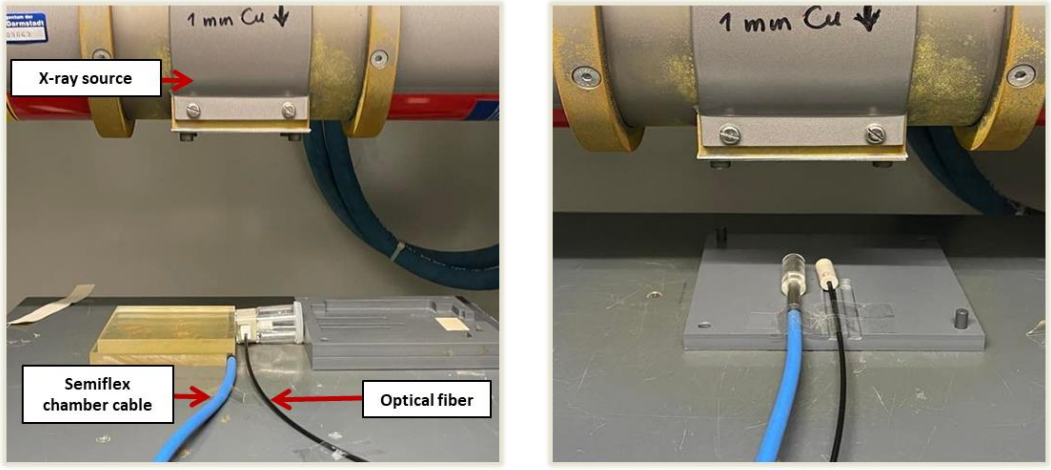

Figure 2 Set up of GSI X-ray machine for irradiation with a 250 kV photon. The dose dosimetry was carried out by the Semiflex chamber (IC, type number TM31013, PTW, Germany). (a) The dosimeter was placed under a Polymethyl Methacrylate (PMMA) slab beside the main container. (b) The dosimeter was enclosed with the dosimeter's build-up cap and placed beside the cylindrical container

3.5 Statistical analysis

Values of oxygen depletion are presented as the average ± standard deviation (SD) of the sixteen, seven, seven and three replicated experiments in full oxygenated water, deoxygenated water, PBS and cell culture medium, respectively. Statistical analysis was performed using Microsoft Excel (version: Microsoft 365 Apps for enterprise) and GraphPad Prism Software (version 8.02; GraphPad Software, Inc.). The One-Way ANOVA test was used to calculate the significance between four sample groups. P-values lower than 0.05 were regarded to indicate a statistically significant difference.

4. Results and Discussion

The average oxygen concentrations in the samples before the irradiation in full oxygenated water, deoxygenated water, PBS, and cell culture medium were 283.87, 207.42, 290.46, and 224.22 µM, respectively. All samples were irradiated into three steps with a dose of 50 Gy per step. The oxygen concentration was measured and plotted during three consecutive irradiations as shown in Figure 3.

In Figure 4 and Table 1, we showed the relation between the oxygen depletion against the cumulative dose. It was found that the amount of oxygen depletion was highest at the first irradiation and then decreased in the steps afterward. In the case of the full oxygenated water, the average oxygen depletion per dose in the first three steps were 0.28, 0.25 and 0.23 µM/Gy. For the deoxygenated water, the average oxygen depletion per dose was 0.26, 0.22 and 0.18 µM/Gy, respectively. The average oxygen depletion per dose in PBS was 0.26 µM/Gy in the first step and then decreases to close to the full oxygenated water with values of 0.26 and 0.23 µM/Gy in the second and third steps of irradiation. The cell culture medium showed the average oxygen depletion per dose were 0.42, 0.38 and 0.37 µM/Gy in the first, second and third steps of irradiation, respectively. According to our findings, the oxygen depletion was significantly observed in culture medium when compared with full oxygenated water ($P < 0.05$). It is consistent with the previous study in which the oxygen depletion in the cell medium was greater than that in buffered water (Evans, 1969; Whillans & Rauth, 1980). Apparently, the oxygen depletion in the cell culture medium was the highest in all three steps of

irradiation. We considered that the largest oxygen depletion in cell culture medium was due to the presence of several organic molecules such as fatty acids and amino acids. The reaction between the organic molecules and other radicals is possible, such as the reaction with hydroxyl radicals ($OH^{\bullet}$). $OH^{\bullet}$ changes the organic molecule to the organic radical $R^{\bullet} + H_2O$ that is capable of reacting with oxygen (Boscolo, Scifoni, Durante, Krämer, & Fuss, 2021).

In general, each step of irradiation, solvated electrons (e_{aq}^-) and hydrogen radicals ($H^{\bullet}$) are created. These are two main species that can interact with oxygen molecules and transform into the superoxide ($O_2^{\bullet-}$) and perhydroxyl radicals ($HO_2^{\bullet}$) (Boscolo et al., 2020). This mechanism results in oxygen depletion in the irradiated samples. For the second irradiation step, there are possibilities that the newly created solvated electrons (e_{aq}^-) and hydrogen radicals ($H^{\bullet}$) can interact with not only oxygen molecules but also the previously formed superoxide ($O_2^{\bullet-}$) and perhydroxyl radicals ($HO_2^{\bullet}$). Therefore, the amount of oxygen molecules depleted in the second irradiation step is lower than it was in the first. The same is true for the third irradiation step. It is clearly seen that our experiments have confirmed the above assumption.

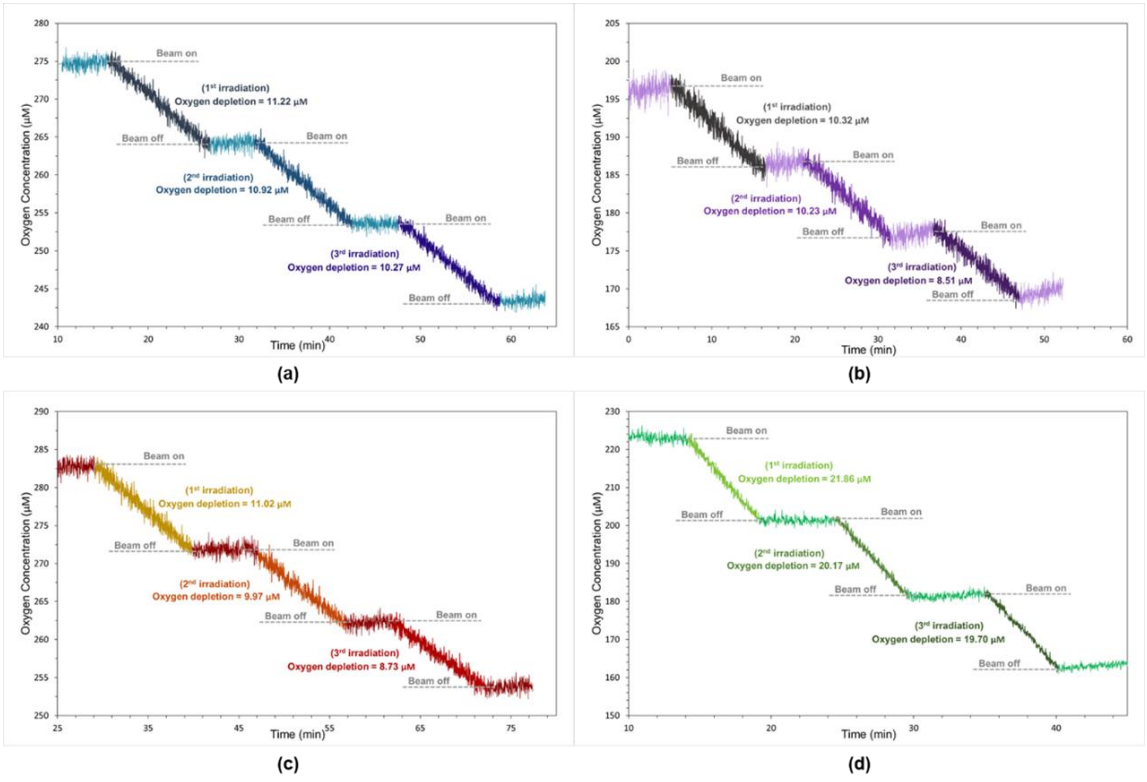

Figure 3 Example curves of the oxygen concentration during three irradiations: (a) full oxygenated water, (b) deoxygenated water, (c) PBS, and (D) cell culture medium. Samples were irradiated for three steps with 50 Gy/step

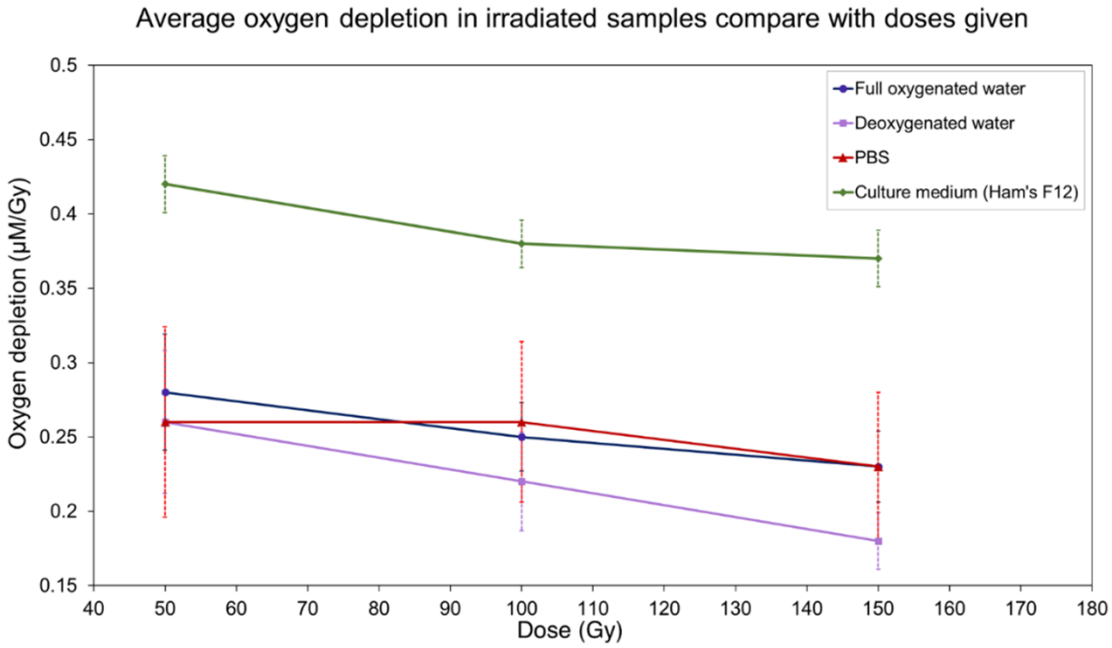

Figure 4 Comparison of the average oxygen depletion against the cumulative doses. In this graph, each line has three points. The first point represents the average oxygen depletion when the sample was irradiated with a dose of 50 Gy. The second and third points represent the average oxygen depletion during the second and third steps of irradiation (50 Gy)

Table 1 Average oxygen depletion in irradiated samples compared to the cumulative doses

Cumulative doses (Gy)	Oxygen depletion (µM/Gy)			
	Full oxygenated water	Deoxygenated water	PBS	Ham's F12
50	0.28 ± 0.039	0.26 ± 0.048	0.26 ± 0.064	0.42 ± 0.019
100	0.25 ± 0.023	0.22 ± 0.033	0.26 ± 0.054	0.38 ± 0.016
150	0.23 ± 0.024	0.18 ± 0.019	0.23 ± 0.050	0.37 ± 0.019

5. Conclusion

The investigation of oxygen depletion during conventional (CONV) irradiation utilizing a conventional dose rate had been performed. Different samples have been irradiated with an X-ray beam at a dose of 50 Gy/step. The experimental results confirmed that the oxygen concentration in all samples decreased during the X-ray irradiation. The data on oxygen depletion was kept as a reference for comparison with the results of the experiment with laser-accelerated electron irradiation in the future.

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