IORT AQURE IN FLASH MODE: DOSIMETRIC CONTROL IN BREAST CANCER CELL *IN VITRO* EXPERIMENTS

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Abstract

Ultra-high-dose rate (FLASH) radiotherapy is a promising cancer treatment method in which high doses of radiation are delivered in a very short time, minimising damage to healthy tissue while effectively targeting tumour cells. In this study, the IORT AQURE accelerator was used in FLASH mode to irradiate breast cancer cells. Dosimetric verification was carried out to confirm the quality of the beam used in the study and to control the doses (5, 10, and 15 Gy) administered to the cells. Gamma index analysis confirmed the accuracy of dose distribution, with results exceeding 96% for all cell samples. Radiobiological testing demonstrated a 90% reduction in the viability of HCC38 breast cancer cells at a dose of 15 Gy. These results validate the film dosimetry for controlling the beam and doses and the use of the AQURE accelerator in the FLASH mode for preclinical research and confirm its potential for future preclinical studies and clinical applications.

Keywords: FLASH RT, ultra-high dose rate, FLASH electron measurements, cell irradiation.

1. Introduction

Cancer remains one of the leading causes of premature death worldwide, second only to cardiovascular diseases. The primary methods for combating cancer include surgery, chemotherapy, and radiation therapy. Among these, radiation therapy continues to evolve, with research leading to new and improved techniques. One such innovative approach is FLASH radiotherapy which involves the use of ultra-high dose rates of ionising radiation, typically exceeding 40 Gy/s, which is considerably higher than those used in conventional radiotherapy (<0.1 Gy/s) [1]. Unlike conventional radiotherapy, which delivers radiation at a steady rate over a longer period [2], FLASH radiotherapy administers a high dose of radiation in an extremely short time, typically less than 200 milliseconds. This rapid delivery of radiation is just as effective as conventional dose rate delivery in destroying cancerous tissues while significantly sparing healthy tissues from damage.

The protective effect on healthy tissues was first observed in the 1950s and 1960s. Early research indicated that non-cancerous mammalian cells exposed to ultra-high radiation dose rates exhibited greater viability than those exposed to conventional dose rates [3]. The efficacy of FLASH therapy in anti-cancer treatment has been confirmed *in vitro* studies in various cell lines [4], such as human lung fibroblast cells [5], human cervical cancer cell lines [6], and human colon cancer cell lines [6], as well as *in vivo* in human and animal skin [7–9] and brain tissue [10–12]. However, there is a paucity of data on the effect of FLASH electron irradiation on human breast cancer cells.

However, it was not until more recent studies that the potential clinical benefits of this effect were more clearly understood and documented. A pivotal study demonstrated this effect by comparing the outcomes of thoracic irradiation in mice. Mice subjected to a single fraction of 17 Gy at conventional dose rates (0.03 Gy/s) developed moderate to severe pulmonary fibrosis within 36 weeks after irradiation. In contrast, when the same dose was administered using an ultra-high dose rate beam (40-60 Gy/s), the incidence of pulmonary fibrosis was significantly reduced [13]. FLASH radiotherapy has shown promising results, with the first clinical trials (Phases I and II) demonstrating a rapid and complete tumour response in a patient with cutaneous lymphoma [8, 14]. Although these findings, along with preclinical and radiobiological studies, support its potential, further preclinical research is still needed to confirm its safety and efficacy before widespread clinical application.

The use of high dose rate beams is limited by significant challenges, such as the dosimetry of ultra-high-dose rate radiation. The primary and secondary standards for the absorbed dose for electron beams and their methodology are well known for conventional radiotherapy beams [15]. However, there are no established guidelines and no specific dosimetry methods for ultra-high dose rates. Passive dosimetry methods are currently used for both research and clinical purposes [16]. *Thermoluminescence dosimetry* (TLD), *optically stimulated luminescence dosimetry* (OSLD), and alanine dosimetry are the most commonly used [17]. The disadvantages of TLD, OSLD, and alanine are that they can only provide point measurements. For this reason, one of the most widely used passive methods is film dosimetry, which does not have such limitations and whose methodology allows for reliable results. The most widely used radiochromic films are Gafchromic Films (Ashland, Wilmington, DE) type EBT-3 or EBT-XD, which provide dose measurements with a dynamic range of up to 60 Gy [8, 9, 17, 18]. An advantage of radiochromic films is the ability to provide either planar or 3D measurements, as well as energy and dose rate independence, which has been confirmed for dose rate measurements up to 410^9 Gy/s [19]. The use of films requires precise calibration and compliance procedures [20]. There are also no dedicated methods of real-time beam monitoring and control. There are no dedicated methods for real-time measurement of the FLASH beam, so another challenge of ultra-high-dose rate beam dosimetry is its monitoring and control. Therefore, work is underway to use modified ionisation chambers, scintillators, and beam-current transformers (BCTs) for this application [21–24].

Another challenge is the availability of beam generation facilities in FLASH. Most of those used for research are modified medical linear accelerators [25]. Electron accelerators for intraoperative radiotherapy (IORT) were the first to be adapted and used in preclinical and clinical studies. FLASH radiotherapy reduces the inconvenience for the patient of having to undergo several weeks of conventional radiotherapy. However, specific preclinical and clinical studies are needed to investigate the use of FLASH beams to target cancer cells effectively.

The results presented in this paper demonstrate the control and verification of the FLASH radiotherapy beam and irradiation fields, confirming the dosimetric accuracy of an ultra-high-dose rate (150 Gy/s) electron beam generated by an intraoperative accelerator. Preliminary radiobiological experiments further illustrate the potential effectiveness of the beam in reducing the viability of breast cancer cells in vitro, indicating its suitability for preclinical applications.

2. Materials and methods

2.1. Accelerator for FLASH research

The ultra-high dose rate (FLASH) mode was achieved with the AQURE intraoperative accelerator, which is intended for use in intraoperative radiotherapy [26]. It is a radiotherapy device that produces ionising radiation: electron beams with energies ranging from 4 to 12 MeV. For standard intraoperative radiotherapy, the maximum conventional dose rate is 10 Gy/min. The conventional intraoperative beam has been validated in radiobiological research [27]. The AQURE has been upgraded to FLASH mode, which generates an ultra-high dose rate beam with two available beam energies: 6 and 9 MeV [24]. Monte Carlo simulations were compared with the measured results of the FLASH beams at dose rates of 300 Gy/s and 440 Gy/s, respectively, confirming that the upgraded intraoperative accelerator can generate high-quality ultra-high dose rate beams dedicated to experiments (FLASH-RT) [28].

The source of electrons accelerated in an accelerating structure is the 15 kV electron gun controlled by the gun modulator. Electrons are shot into the structure with an initial energy of several keV and accelerated in the electromagnetic field. In the FLASH mode of the AQURE accelerator, the dedicated programme allows for the emission of single pulses with widths of 2 to 5 µs. Consecutive pulses can be triggered with frequencies from 1 to 300 Hz. The outgoing beam is shaped by a scattering foil, a flattening filter and finally by applicators [29]. In this way, the electron beam provided by the accelerator is flat and symmetric, allowing the irradiation of a field with a diameter of 4 to 10 cm. The AQURE FLASH device is mobile, allowing the head to be tilted from/to the column in the range of 45° and 30° and the head to be tilted laterally by ±45°. In the studies presented here, an electron beam with an energy of 9 MeV and an ultra-high dose rate of 150 Gy/s (1 Gy/pulse) was used.

2.2. Film dosimetry for the FLASH beam

For FLASH beam measurements, we used film dosimetry that applies self-developing radiochromic films. After electron irradiation, the films change colour of the polymers, allowing the dose received to be quantified from the measured optical density. The observed colour change is usually recorded using flatbed optical scanners [16].

Film dosimetry using Gafchromic EBT-XD films was used for all dosimetric measurements, *i.e.*, depth and spatial dose distributions. The EBT-XD films were placed in the phantom RW3 plate. To obtain a percentage depth dose distribution, the film was placed parallel to the beam in the centre of the irradiation field. The electron beam profile was obtained by placing the film perpendicular to the beam. PDD and profile measurements were performed at a *source-to-skin distance* (SSD) equal to 60 cm using a PMMA applicator with a diameter of 100 mm.

The same type of Gafchromic films was also used to evaluate the homogeneity and repeatability of the irradiation field of the biological material. The films were placed under flasks, so it was necessary to introduce a correction factor for the attenuation of the beam after passing through the medium. All measurements were performed at an SSD of 60 cm. A dosimetric film was placed under each irradiated flask to compare the dose distributions for each biological sample. Figure 1 shows a schematic diagram of the measurement system, where we marked a Gafchromic film under the flask with cells in medium. The flask was placed under a PMMA applicator with a diameter of 100 mm.

The thickness of the medium in the flask was the same for each irradiation in order not to introduce dose error due to the passage of the beam and the deposition of the dose in different thicknesses of the medium. Therefore, a constant amount of medium, as small as possible, was
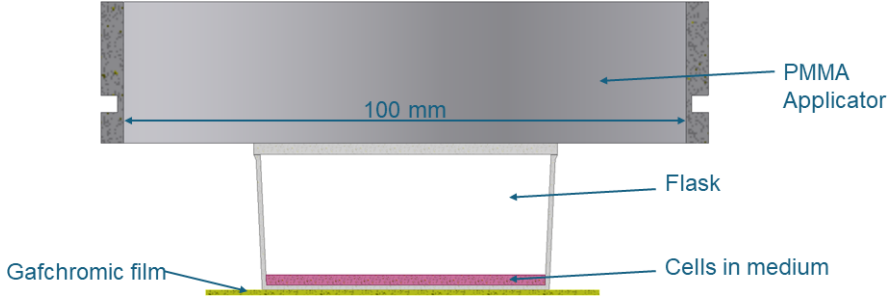
Fig. 1. Diagram of the irradiation measurement system stand. The Gafchromic film was placed under a flask of biological material under the PMMA applicator with a diameter of 100 mm and the Gafchromic film for dose distribution measurements.

used. Before irradiation, the negligible effect of the medium thickness on the deposition dose was checked and confirmed.

Twenty-four hours after irradiation, the films were scanned with the Perfection V850 Pro flatbed scanner (Epson, Long Beach, CA, USA) at 72 dpi resolution and analysed using a calibration curve prepared for a conventional electron beam of 9 MeV using MEPHYSTO mc^{2}software (version 1.8) by PTW (PTW, Freiburg, Germany).

2.3. Gamma index analysis

To compare the field distribution under the irradiated vials with the biological material, the position of the vial on the film was marked. This allowed the same reproducible region of interest to be defined for each sample taken for analysis. A 2D gamma (γ) index analysis was performed using Verisoft software (version 6.0) by PTW. The gamma index allows for a point-by-point comparison of differences between two dose distributions in terms of both *distance to agreement* (DTA) (ΔD_d) and *dose difference* (DD) (Δd_d) [30]. The dose distributions given for the analyses are described by the parameters: distance (r_a) between the points for which the dose (D_a) is determined and the dose (D_a). In clinical practice, acceptance criteria are most often defined as 3 mm (DTA) and 3% (DD). This means that the same point in two dose distributions must not differ by more than 3% in dose value and must not be more than 3 mm apart (the criteria is 3mm/3%) [31]. To make a comparison, it is necessary to determine a reference distribution, *i.e.*, the point (r_r) receiving the dose D_r. The ellipsoid defined by (1) describes the representation of the acceptance criterion determination method, considering both the DD and DTA criteria [30].

$$1 = \sqrt{\frac{\Delta r^2}{\Delta d_d^2} + \frac{\Delta D^2}{\Delta D_d^2}} \tag{1}$$

where: $\Delta r = |r_r - r_a|$; $\Delta D = |D_a(r_a) - D_r(r_r)|$.

To adjust the reference dose measurement at point r_r for the compared dose distribution, it is necessary to determine at least one point r_a, D_a) located inside the acceptance ellipsoid for which:

$$\Gamma(r_a, D_a) = \sqrt{\frac{\Delta r^2}{\Delta d_a^2} + \frac{\Delta D^2}{\Delta D_a^2}} \leqslant 1. \tag{2}$$

The minimum of $\Gamma(r_a, D_a)$ is defined as the quality factor (r_r) for the reference point r_r. When the factor value is <1, the criterion is met. The analysis is performed for each point in

the compared area [32]. Determining the number of points from the compared distributions that should meet the acceptance criteria is necessary to evaluate the results of the analyses. In gamma analysis used in *intensity-modulated radiation therapy* (IMRT), dose distributions are considered satisfactory when 95% of the points meet the acceptance criteria [33].

Gamma analyses were performed to compare the results for each sample for every dose administered (5 Gy, 10 Gy, and 15 Gy). The gamma index was performed for the criteria $\Delta D_d = 3$ mm and $\Delta d_d = 3\%$ or $\Delta d_d = 1\%$. This was achieved by selecting dose distribution for the first sample of each dose in the experiment as the reference and then comparing it using gamma analysis.

2.4. Cell culture

Human breast cancer HCC38 cells (ATCC® CRL-2314TM) were used to study the effectiveness of electron beam quality. These cells were cultured in RPMI 1640 medium with 10% foetal bovine serum and 1% antibiotic-antimycotic solution (all from Life Technologies, USA). The media changed three times per week. HCC38 cells were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2 until approximately 80% confluence.

2.5. Cell irradiation

The HCC38 cells used in the experiment were seeded 24 hours before irradiation at a density of $4 \cdot 10^3$ cells per cm^2 in 25 cm^2 flasks (in passages 5-6). The cells were exposed to three different doses of radiation, 5 Gy, 10 Gy, and 15 Gy, in flasks containing 5 ml of medium. The irradiation was performed at SSD = 60 cm using the PMMA applicator, which limited the irradiation field to a circle of 100 mm diameter. For each experiment, control cells, which remained in the incubator, and sham control cells, which were exposed to all environmental factors as the treated cells but without irradiation, were included. Before the experiment, the medium in the flasks was replaced for both control and irradiated cells.

2.6. Cell viability and morphology

Cell morphology and number of changes were observed under a light microscope (Primo Vert, Zeiss, Germany) using 10x objectives. Microscopic observations were performed daily from Day 1 to Day 7 after irradiation. On the seventh day after irradiation, cells were stained with the LIVE/DEAD ®Viability/Cytotoxicity Kit for mammalian cells (Invitrogen™, USA) to assess the integrity of the membrane cells remaining on the plate. The observation was performed with confocal laser scanning microscopes (LSM 700, Zeiss, Germany) at an excitation wavelength of 485 nm for calcein (live cells) and 530 nm for ethidium homodimer-1 (dead cells). A quantitative cell viability assay was performed using PrestoBlue™Cell Viability Reagent (Life Technologies, USA). Cells were treated with 10% PrestoBlue reagent solution in the medium for 2.5 hours and then transferred to a 6-well plate. Fluorescence intensity was measured using a SpectraMax i3x plate reader (Molecular Devices, CA) at a wavelength of $\lambda_{ex}560/\lambda_{em}590$. Results are expressed relative to the sham control as a percentage of viable cells. Statistical analysis was performed using GraphPad Prism 10 (version 10.1.2 for Windows, GraphPad Software). One-way analysis of variance (ANOVA) with 's multiple Šidák comparison test was used.

3. Results

To prepare the beam for irradiation and confirm the dosimetric parameters of the ultra-high-dose rate electron beam, the depth distribution and dose profiles were performed using a PMMA applicator with a diameter of 100 mm. The nominal energy of the used electron beam was 9 MeV, which confirms the percentage dose depth curve and the dosimetric parameters presented in Table 1. For the 9 MeV FLASH beam, the most probable energy at the phantom surface is $E_p = 10.1$ MeV, and the mean energy at the phantom surface is $E_0 = 9.1$ MeV.

The uncertainties of the depth-dose parameters (R100, R90, R80, R50, R_p) were estimated by combining the known dose uncertainty of the Gafchromic EBT-XD film ($\sim$ 2%) with the local dose gradient of the PDD curve. Depth uncertainty was then approximated by dividing the dose uncertainty by the slope of the PDD at each relevant point. For R100, which lies in the high-dose plateau region with minimal gradient, a higher uncertainty was assumed to reflect reduced precision in locating this point [34].

Table 1. Percentage depth dose distribution parameters, *i.e.*, depths of 100%, 90%, 80%, and 50% dose (R_{100}, R_{90}, R_{80}, R_{50}), and the depth of practical range R_P for FLASH beams of 9 MeV nominal energy at 150 Gy/s dose rate obtained from film dosimetry measurements.

Parameter	Depth [mm]
R_{100}	10.50 ± 0.5
R_{90}	24.89 ± 0.22
R_{80}	29.56 ± 0.29
R_{50}	48.89 ± 0.57
R_P	49.54 ± 1.0

To determine the symmetry and homogeneity of the FLASH beam, the cross-plane distribution of the beam was also measured. For medical accelerators, International Electrotechnical Commission (IEC) standards define beam symmetry as the maximum dose ratio at points symmetrically distant from the beam axis in an area 1 cm smaller than the field determined by the 90% isodose, and for the beam used in this study, it was $1.93\% \pm 0.71\%$. The beam flatness is defined by IEC standards as the maximum percentage difference between the maximum and minimum dose over the area covered by the 90% isodose minus 1 cm, and for the beam used in this study, it was $1.55\% \pm 0.88\%$. Figure 2 shows the PDD curve (a) and the profile (b) of the FLASH 9 MeV electron beam at 150 Gy/s dose rate. The apparent increase in dose at approximately 67 mm depth (Fig. 2a) is most likely due to film-based measurement artefacts, such as scanner backscatter, optical noise, or film nonlinearity at low dose regions. Since this feature appears beyond the clinically relevant range and involves a small percentage of the maximum dose, it does not affect the interpretation of the therapeutic depth or dose distribution. Although uncertainties were estimated for symmetry and flatness, they are not displayed as error bars on the PDD and profile plots in Fig. 2. This is a common approach in film dosimetry studies due to very high spatial resolution and the large number of dose points, which would make graphical representation of uncertainties impractical and visually cluttered.

Evaluation of the irradiated field with film dosimetry was achieved by using radiochromic films placed under the flasks containing adherent cancer cells during irradiation. Table 2 presents the results of the gamma analysis obtained for each dose (5 Gy, 10 Gy, and 15 Gy) delivered to biological materials at ultra-high rates (150 Gy/s). This was measured on three independent biological samples exposed to each of the above doses. In the gamma analysis, Sample I was defined as the reference for each dose.
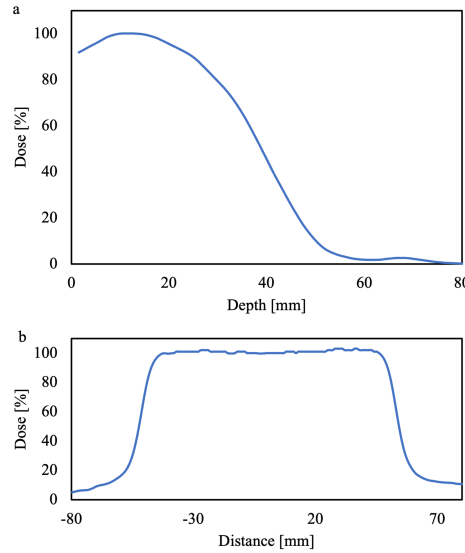
Fig. 2. Percentage dose depth curve (a) and cross-plane profile (b) of a 9 MeV FLASH electron beam at a dose rate of 150 Gy/s obtained from film dosimetry measurements.

Table 2. Results of gamma analysis for dose distributions among irradiated vials containing biological material. The gamma index analysis compared samples for each dose, using sample number I as a reference.

Dose [Gy]	Sample number	Gamma index [%]	
		3 mm/3%	3 mm/1%
5	I	100	100
	II	99.7 ± 0.038	97.4 ± 0.112
	III	99.8 ± 0.031	97.2 ± 0.116
10	I	100	100
	II	99.9 ± 0.022	96.1 ± 0.136
	III	99.5 ± 0.050	96.7 ± 0.126
15	I	100	100
	II	99.2 ± 0.062	98.0 ± 0
	III	97.7 ± 0.105	96.5 ± 0

Dosimetric measurement of dose distributions using a radiochromic film placed under the flasks with the cancer cells shows slight differences in the doses deposited in the biological material. Gamma analysis for 3 mm/3% and 3 mm/1% acceptance criteria was performed to check and eliminate significant differences in dose heterogeneities and errors. The differences between the gamma index results for the 3 mm/3% and 3 mm/1% parameters did not exceed 4%. The gamma index analysis was carried out for the two acceptance criteria to give a more detailed indication of possible local differences in dose distribution for the high doses used in the experiment, which can be as high as 0.45 Gy for the 3 mm/3% acceptance criterion, and which could affect the biological results. The uncertainty of the gamma analysis results was estimated based on binomial statistics, treating each evaluated dose point as an independent pass or fail outcome. The resulting statistical uncertainty of the reported agreement percentage depends on the total number of analysed points and was calculated following the method proposed by Cutanda Henríquez and Vargas Castrillón [35].

Figure 3 shows a map of the gamma index analysis for the 3 mm distance-to-agreement and 1% dose difference criteria for dose distribution under irradiation cuvettes with a dose of 15 Gy, comparing Samples I and II (3A) and Samples I and III (3B) with criteria 3 mm/1% and comparing Samples I and II (3C) and Samples I and III (3D) for criteria 3 mm/3%, according to the results in Table 2. The yellow dots in Fig. 3 represent areas where the gamma index was greater than 1%, appearing mostly at the periphery of the analysis area. Minor visual discrepancies between maps with different criteria, especially at the field edges, may result from the applied 5% dose threshold and exclusion of low-dose pixels from the gamma analysis.
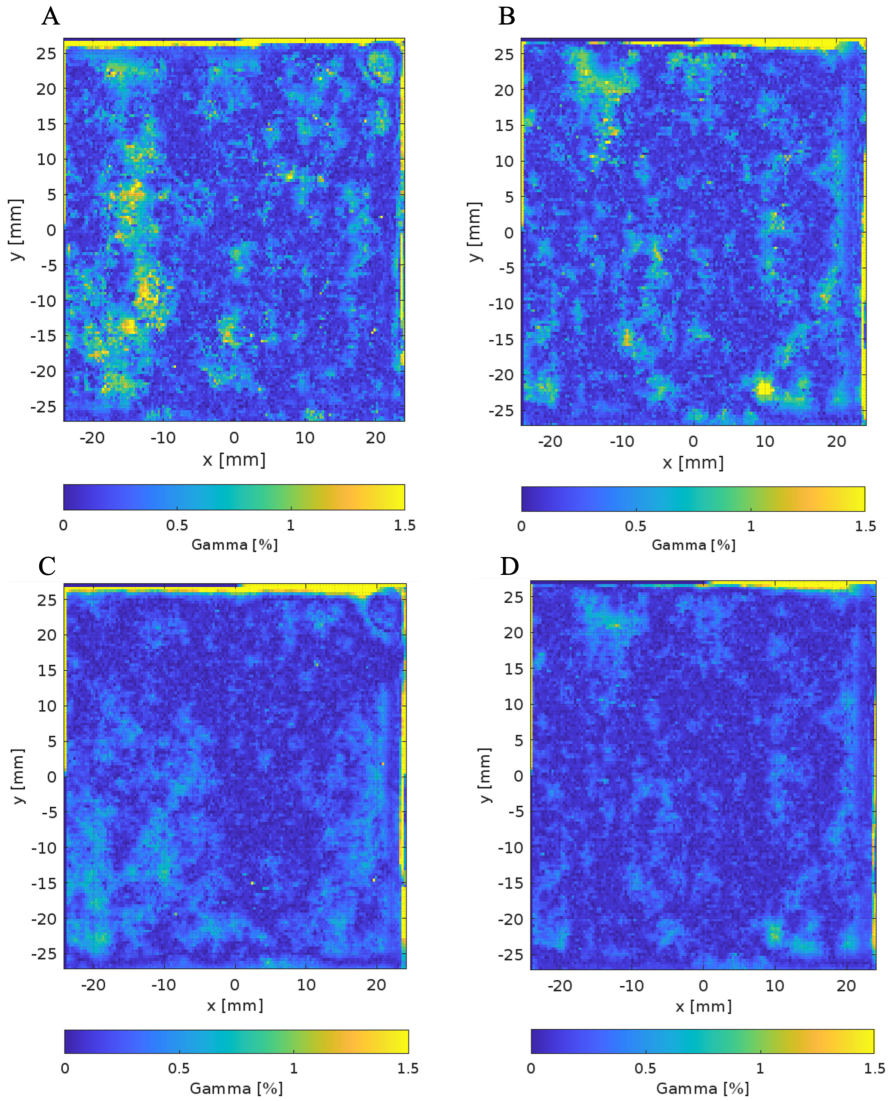

Fig. 3. Gamma analysis for 15 Gy irradiation fields. The first dose distribution (Sample I) is defined as the reference, with comparisons made between Sample I and Sample II (A) and between Sample I and Sample III (B) for criteria 3 mm/1% and comparisons between Sample I and Sample II (C) and between Sample I and Sample III (D) for criteria 3 mm/3%.

Under the conditions described, the effects of irradiation on breast cancer HCC38 cells were assayed and cell viability was evaluated. To exclude the influence of the external environment on cell viability, sham samples were also analysed. These samples were processed in the same way as the exposed ones, but were not irradiated.

Figure 4 shows the effect of the radiation dose on the viability of breast cancer cells on Day 7 after exposure to radiation at doses of 5, 10, and 15 Gy. FLASH irradiation caused a significant decrease in cell viability by 75%, 88%, and 90% compared to the control samples at doses of 5, 10 and 15 Gy, respectively (Fig. 4a). No changes were found between the control and sham samples, which excludes the influence of other external factors (*e.g.*, transport in a portable incubator) on the viability of the cells tested in the experiments. The best fit of the trend line versus the decrease in cell viability concerning the radiation dose is described by a power law relationship (R= 0.996) (data not shown).
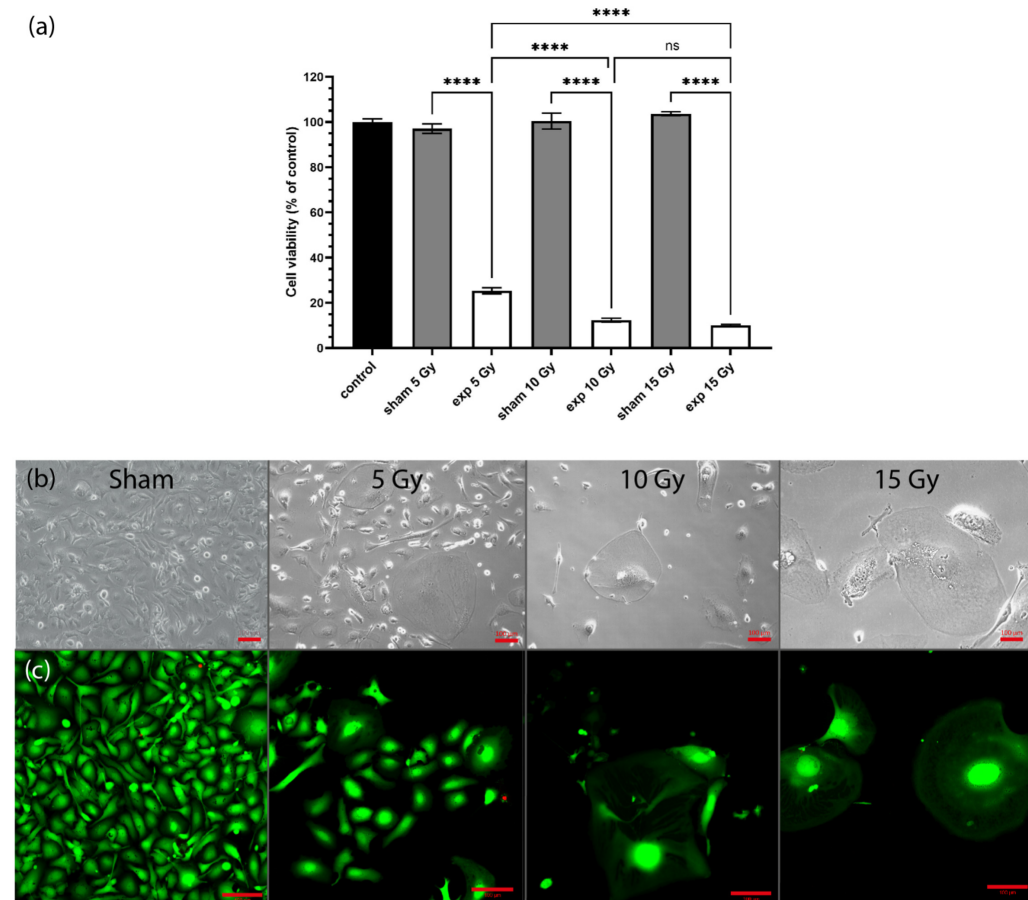

Fig. 4. Viability and morphology of HCC38 ce0xlls from breast cancer 7 days after FLASH irradiation at doses of 5 Gy, 10 Gy, and 15 Gy. Metabolic activity (viability) of cells in control, sham, and exposed (exp.) samples determined by the Presto Blue assay (a); morphologies of cells remaining on the plates without and after irradiation as observed by optical (b) and CLSM (c) microscopy. The LIVE/DEAD™Viability/Cytotoxicity Kit was used to assess the viability of the cells remaining on the plates – green cells with intact membranes are alive and red cells with damaged membranes are dead (c). The images were taken with a 10×objective, with the magnification indicated by a scale bar (100 µm). Statistical significance is indicated by asterisks (**** p <0.0001), while "ns" denotes a non-significant difference (p ⩾ 0.05).

The significant reduction in breast cancer cell numbers on Day 7 after FLASH irradiation was also visible through microscopic observation. In addition, changes in the morphology of irradiated cells were more evident with increasing doses. After irradiation, larger and more rounded cells were observed (Figs. 4b and c). The cells remaining on the plate after exposure to FLASH irradiation were also found not to have damaged cell membranes and were alive (Fig. 4c).

4. Discussion

Currently, there are no devices available on the market for clinical use of a FLASH beam. The available options are either research-only devices or modified medical accelerators [23]. A device dedicated to FLASH radiation should meet the parameters of FLASH radiotherapy, precisely delivering a high dose in a short time (<200 ms) with a dose rate greater than 40 Gy/s [36]. The beam used in this study was generated by the AQURE-FLASH device with FLASH parameters. The dose rate of the 9 MeV FLASH beam used in this study was 150 Gy/s, and the doses were delivered at a maximum of 200 ms for the dose of 15 Gy, and the dose in a pulse was 1 Gy/pulse. Measurement of the percentage dose depth distribution confirms the beam energy. The distribution of the dose profile shows the symmetry and homogeneity of the electron fields according to the IEC standards [37,38].

The results of the comparison of the dose distributions of irradiated samples with gamma analysis (using the 3mm/1% criteria) yielded an agreement of more than 96%, which is considered satisfactory, considering the requirements for other clinically used techniques such as IMRT [33]. The results indicate that these differences are not due to differences in the deposited dose but to placement errors. This could, in turn, be due to a placement error in the definition of the analysis area or inhomogeneity of the Gafchromic film after being cut to the size and shape of the flask. Since it is difficult to achieve reproducibility in positioning, scanning and reading the dose distribution under the flasks, the placement error can be defined as 3 mm. However, these errors do not affect the quality of the irradiation fields of biological material, as the results of the gamma index analysis for each sample are greater than 96%, so the irradiation fields are reproducible, which is essential in biological experiments. This outcome supports the conclusion that the dose distributions are homogeneous across samples, thereby confirming the biological findings. However, it is important to note that the use of this passive method introduces a time delay in obtaining real-time feedback on the delivered doses and their distribution within the experimental samples [17].

In this paper, we also demonstrated the biological efficacy of ultra-high-dose FLASH rates of 150 Gy/s by reducing the viability of human breast cancer cells at three different doses. The percentage of viable cells was determined using a PrestoBlue assay, which was used to examine the metabolic activity of cancer cells in both non-irradiated controls and after exposure to consecutive doses of FLASH irradiation. Adrian *et al.* examined the viability of seven cell lines, including two breast cancer lines (MCF-7 and MDA-MB-231), under the influence of FLASH at doses of approximately 3, 6, and 9 Gy. The results showed that increasing the dose from 3 to 9 Gy resulted in a decrease in the survival fraction of MCF-7 and MDA-MB-231 breast cancer cells [6], similar to the decrease observed in the HCC38 line studied here. The results clearly showed a significant decrease in the viability of HCC38 cells at doses ranging from 5 Gy to 10 Gy compared to the sham control, seven days after exposure. However, in this study, an even higher dose of 15 Gy was used, but the decrease in cell viability was not statistically significant compared to the radiation of 10 Gy. We also observed that the morphology of the cells remaining on the plate changed after exposure. The cells became larger and rounder, suggesting the effect of cellular senescence seen with cellular radiotherapy. Induction of senescence in cancer cells has recently been shown to also be one of the fundamental mechanisms of the anti-cancer activity of radiotherapy [39].

5. Summary

This work presents the dosimetry results of an ultra-high dose rate electron beam (FLASH) obtained by an intraoperative accelerator. The AQURE-FLASH was used for the first time for cell viability research, and the results showed a decrease in HCC38 cancer cell viability with an increase in the delivered dose via an ultra-high dose rate beam.

The results demonstrated the feasibility of effective control of the doses delivered and of monitoring of the irradiated sample dose distributions using film dosimetry in radiobiological experiments. The dosimetry results showed a high-quality beam with an energy of 9 MeV at a dose rate of 150 Gy/s. The beam is symmetrical and homogeneous according to the IEC standards for electron beams in medical accelerators (flatness – 1,55% and symmetry – 1,93%) for an irradiation field of 100 mm diameter. Repeatability of dose distributions compared with gamma index analysis showed that at least 96% of the points taken for analysis differ from each other by up to 1% of the delivered dose in a distance of 3 mm.

The study also highlights the effectiveness of the upgraded AQURE accelerator beam in destroying cancer cells, with the greatest reduction in HCC38 cell viability observed at a dose of 15 Gy, resulting in a 90% decrease compared to the control. More extensive in vitro biological studies on healthy and cancer cells are planned to better understand the differences in cell irradiation using the FLASH method compared to conventional radiotherapy.

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