A High-Throughput In Vitro Radiobiology Platform for Megavoltage Photon Linear Accelerator Studies

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1. Introduction

Radiotherapy is an important cancer treatment modality as more than half of cancer patients will require some form of radiotherapy during their illness [1]. Preclinical radiobiology experiments can provide valuable information about tumours and normal tissue radiation effects at the molecular level and serve as a framework to study radiation–drug combinations. These preclinical explorations may assist in developing clinical trial strategies, which can ultimately improve the outcomes of radiotherapy treatments.

Various approaches have been used to improve the therapeutic ratio in radiotherapy [2]. A large number of novel anticancer agents targeting cancer cell signalling pathways and several immunotherapy agents are now in routine clinical use. Combining these novel agents with radiation offers many therapeutic opportunities [3]. The challenge is to suggest optimal clinical radiation–drug schedules given a large number of potential combinations, doses, and schedules of each agent.

Preclinical models can help to explore this large experimental space of radiation–drug combinations, but each of them has its advantages and disadvantages [4]. In vitro models and assays are commonly employed for the preliminary screening [5,6], and large tissue culture vessels and laboratory irradiators are widely used for this purpose [7]. These allow

Expected Application: Preliminary in vitro screening of radiation–drug combinations within a clinical set-up.

Abstract: We designed and developed a multiwell tissue culture plate irradiation setup, and intensity modulated radiotherapy plans were generated for 96-, 24-, and 6-well tissue culture plates. We demonstrated concordance between planned and measured/imaged radiation dose profiles using radiochromic film, a 2D ion chamber array, and an electronic portal-imaging device. Cell viability, clonogenic potential, and γ-H2AX foci analyses showed no significant differences between intensity-modulated radiotherapy and open-field, homogeneous irradiations. This novel platform may help to expedite radiobiology experiments within a clinical environment and may be used for wide-ranging ex vivo radiobiology applications.

Keywords: intensity-modulated radiotherapy; in vitro radiobiology platform; personalised radiotherapy; preclinical radiotherapy studies; radiation-drug combinations

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one to culture large numbers of cells and enable homogeneous radiation dose distributions across the tissue culture vessel. However, their large size may also mean that only a limited number of experimental conditions are possible per tissue culture vessel, which may have resource and cost implications [8]. Furthermore, when multiple tissue culture plates are used for replicate experiments, variations such as differences in incubation and drug exposure times, and exposure to different environmental conditions can lead to unintended variations between experiments. A high-throughput in vitro platform that is efficient, quick, and predictive would help to address some of these challenges.

A high-throughput in vitro platform would help to examine human-derived tumours, which, compared to animal models, avoid variabilities due to interspecies differences, and offer an opportunity to test other cells in the tumour microenvironment, such as vascular endothelial cells, which may be important to the radiation response. Additionally, this platform would enable testing various radiation response modifiers and potentially personalising radiotherapy through mathematical modelling [9].

Intensity-modulated radiotherapy (IMRT) is a well-established clinical radiotherapy technique where the delivery of radiation beams is dynamically modulated to create nonuniform radiation beam intensities. This nonuniform radiation beam profile potentially offers the ability to deliver different radiation doses to a single multiwell tissue culture plate, which may help to expedite the screening of numerous radiation–drug combinations. There are concerns regarding radiation beam homogeneity between and within, individual wells when multiwell tissue culture plates are used due to a lack of full scatter conditions due to air cavities and multiple tissue–air cavity interfaces that potentially introduce variability between experiments.

We aimed to develop and validate a high-throughput in vitro radiobiology platform using IMRT and widely available clinical radiotherapy facilities for conducting radiobiology experiments within a clinical environment and to investigate beam inhomogeneity issues when using multiwell tissue culture plates.

2. Materials and Methods

2.1. Plate Irradiation Setup, Scanning, and Contouring

A tissue culture plate holder was designed and created in a radiotherapy mould room facility using Perspex for its near tissue-equivalent properties and ease of manufacture to irradiate 96-, 24-, and 6-well tissue culture plates (Figure 1A). This holding plate consists of an upper (with two halves) and lower plate with a space created in the middle to hold three types of tissue culture plates composed of polystyrene with varying well volumes (6-well, 3 mL; 24-well, 1 mL; and 96-well, 400 μL) and each with dimensions of 128 × 86 mm, enabling easy handling of the plates without much disturbance of cultured cells during experiments. The dimensions of this holder were kept sufficient to cover full scatter conditions. The holder was able to be attached to the indexed immobilisation positioning system of both a clinical linear accelerator and computed tomography (CT) scanner with a base plate made of medium-density fibreboard (Figure 1B–D). We positioned 96-, 24-, and 6-well tissue culture plates, with wells prefilled with water representing typical amounts of tissue culture media, in a CT scanner (GE LightSpeed, GE Healthcare) with the aid of room lasers (Laser Applikationen, Luvemberg). The plates were scanned (Figure 1B) with an image slice thickness of 1.25 mm for 96- and 24-well tissue culture plates and 2.5 mm for the 6-well tissue culture plate. The images were then transferred to a radiotherapy treatment planning system (Varian Eclipse™, Varian Medical Systems, Inc., Palo Alto, CA, USA) and water columns of individual wells were contoured manually (Figure 2A–F).
Figure 1. (A) Design of multiwell tissue culture plate holding device. The top plate (stone colour) has two halves to facilitate the handling of plates for accurate positioning. The bottom plate is shown in light green and a representative tissue culture plate is shown in red. (B) Multiwell tissue culture plate scanning in a computed tomography scanner. (C,D) Multiwell tissue culture plate irradiation in a clinical linear accelerator. Plates positioned within multiwell tissue culture plate holding device, with the use of room lasers for accurate reproducibility. Plate holder attached to the CT scanner and linear accelerator by a medium-density fibreboard plate (brown).

2.2. IMRT and Open-Field Homogeneous Irradiation Planning

Three individual IMRT plans with target radiation doses of 0, 2, 4, 8, 16, and 24 Gy were generated for the 96-, 24-, and 6-well tissue culture plates using nominal 10MV X-rays. For the 96-and 24-well plate IMRT plans (Figure 2G,H), each column of wells was given a target radiation dose. To achieve a good transition of radiation doses, one column of wells between each target radiation dose level was sacrificed for the 96-well plate IMRT plan. For the 6-well tissue culture plate IMRT plan (Figure 2I), each well was assigned a target radiation dose. Using IMRT inverse planning algorithms, an objective was set to cover 95% of the target volume by the designated target dose to generate IMRT plans. An open-field homogeneous plan was generated for each target radiation dose.
Figure 2. (A–C) Coronal view of 96-, 24-, and 6-well tissue culture plates showing contouring of wells. (D–F) Axial view of 96-, 24-, and 6-well tissue culture plates showing contouring of wells (in 96-well plate contouring, alternate columns of wells were sacrificed during IMRT planning). (G–I) Multiwell tissue culture plate IMRT plans. Contouring are colour-coded. Radiation absolute dose distributions are shown as dose wash (purple—0 Gy, blue—2 Gy, light blue—4 Gy, turquoise—8 Gy, yellow—16 Gy, and red—24 Gy).

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2.3. Verification of the Dose Delivery of IMRT Plans

Verification of accurate delivery of IMRT plans was performed using a scanner (Epson Expression 10000 XL) and pre-calibrated radiochromic films (Gafchromic™ EBT) [10], a 2D ion chamber array (PTW OCTAVIUS Detector 729, Verifisoft v5.1), and an electronic portal imaging device (EPID) (Varian TrueBeam™, Varian Medical Systems, Inc., Palo Alto, CA, USA) [11].

2.4. Cell Lines and Tissue Culture

A melanoma cell line, A375 (American Type Culture Collection, ATCC® CRL-1619™), and a glioma cell line, LN18 (ATCC® CRL-2610™), were grown in Dulbecco’s modified Eagle medium, supplemented with foetal bovine serum (10% for A375 and 5% for LN18), 1% l-glutamine and 1% penicillin–streptomycin (all reagents from Sigma-Aldrich, Gillingham, UK) using standard tissue culture methods.
2.5. Cell Viability

A375 and LN18 cell lines were irradiated in a linear accelerator with the 96-well plate IMRT plan and with open-field, homogeneous irradiations. The cell viability signal (measured at 490 nm) of each radiation dose was assessed with the MTS tetrazolium colorimetric cell viability assay (CellTiter 96® AQueous One, Promega, Madison, WI, USA). In addition, the cell viability signal was calculated using a colorimetric plate reader (VARIOSKAN FLASH, Thermo Scientific, Vantaa, Finland) and analytical software (Skanlt Software 2.4.5, Waltham, MA, USA). The cell viability signal of each radiation dose of the IMRT plan was compared to that of the open-field, homogeneous irradiations (measured from the first six columns of wells).

2.6. Clonogenic Assay

The A375 cell line was seeded with an inoculation density of 500 cells/well in 6-well tissue culture plates. Plates were irradiated with the 6-well plate IMRT plan and with open-field, homogeneous irradiations. Clonogenic assays [12] were performed to assess the clonogenic potential of cells. Plates were scanned after 7–8 days when colonies (a colony was defined as a group of approximately 50 cells, determined by light field microscopy) were formed with ChemiDoc-It™2 imager (UVP, Upland, CA, USA) and colonies were counted using the ImageJ software. The colony number for each radiation dose of the IMRT plan was compared to that of open-field, homogeneous irradiations.

2.7. γ-H2AX Assay

A quantitative method, an In-Cell Western® assay (LI-COR Biotechnology), was used. A375 cell line in 96-well plates were irradiated. Two hours after irradiation, cells were fixed with 4% paraformaldehyde and permeabilised with 0.1% Triton X-100. Cells were stained for phosphorylated γ-H2AX foci with phosphohistone H2AX (Ser 139) rabbit McAb primary antibody (Cell Signalling Technology (#9718S), Danvers, MA, USA) and goat anti-rabbit IRDye secondary antibody (LI-COR Biotechnology, part number 925–3211). Nuclear and cytoplasmic cell staining was performed with CellTag700 (LI-COR Biotechnology, product number 926–41090) for the normalisation of phosphorylated γ-H2AX foci (a surrogate marker for double strand DNA breaks) to the cell number within each well. Plates were imaged with an Odyssey CLx Imager and the γ-H2AX foci signal intensities were measured with Image Studio software (LI-COR Biotechnology).

2.8. Evaluation of Radiation Beam Homogeneity

A375 and LN18 cell lines were irradiated in 96-well plates with open-field, homogeneous 10 MV X-ray irradiations to 2, 4, 8, 16, and 24 Gy in a linear accelerator (Varian TrueBeam™, Varian Medical Systems, Inc., Palo Alto, CA, USA). Irradiations (8 and 24 Gy) were also performed with an orthovoltage unit (Gulmay, Xstrahl) using 250 KV X-rays. The homogeneity of radiation beams was assessed with cell viability assays. The A375 cell line was irradiated to 8 Gy using 250 KV irradiations, and phosphorylated γ-H2AX foci signal levels were analysed to evaluate the homogeneity within wells.

2.9. Statistical Analysis

GraphPad Prism v7 was used to generate graphs and for statistical analysis. Cell viability, clonogenic assay, and γ-H2AX experimental results from the plate IMRT plan irradiations were fitted with linear regression modelling and the results were directly compared with the results of 0, 2, 4, 8, 16, and 24 Gy open-field, homogeneous irradiations. After normal distribution was confirmed (QQ plots, Kolmogorov–Smirnov, and Shapiro–Wilk tests), an unpaired t-test was used to ascertain statistical significance. For radiation beam homogeneity analysis, the average cell viability signal from the central wells was compared to the average viability signal of cells from the edge wells, and a two-way analysis of variance was used for statistical analysis.
3. Results

3.1. Multiwell Tissue Culture Plate IMRT Plans

All three tissue culture plate IMRT plans achieved satisfactory mean doses for each target radiation dose level for the objective set during the IMRT planning process (Table 1). The aim was to cover at least 95% of the target volume by the target radiation dose so that most cells would receive the target dose, while accepting there may be some degree of variation in the mean dose.

Table 1. Radiation dose statistics of multiwell tissue culture plate IMRT plans (radiation doses in Gy).

| Target dose | 96-Well Plate IMRT Plan | 24-Well Plate IMRT Plan | 6-Well Plate IMRT Plan |
|-------------|-------------------------|-------------------------|------------------------|
|             | Mean dose               | Maximum dose            | Minimum dose           | Mean dose               | Maximum dose            | Minimum dose           | Mean dose               | Maximum dose            | Minimum dose           |
| 0           | 0.27                    | 0.46                    | 0.16                   | 0.42                    | 1.76                    | 0.13                   | 0.47                    | 2.85                    | 0.14                   |
| 2           | 2.41                    | 2.7                     | 2.07                   | 2.4                     | 3.59                    | 1.77                   | 2.41                    | 3.6                     | 2                      |
| 4           | 4.38                    | 4.97                    | 3.93                   | 4.48                    | 6.82                    | 3.64                   | 4.56                    | 8.62                    | 3.4                    |
| 8           | 8.57                    | 9.84                    | 7.76                   | 8.71                    | 12.27                   | 7.41                   | 8.74                    | 14.14                   | 6.84                   |
| 16          | 16.96                   | 18.29                   | 15.52                  | 17.37                   | 20.28                   | 15.16                  | 17.34                   | 18.66                   | 14.3                   |
| 24          | 25.2                    | 27.11                   | 22.74                  | 25.54                   | 27.59                   | 21.46                  | 26.08                   | 27.72                   | 22.86                  |

3.2. Dosimetric Verification of Accurate Delivery of Tissue Culture Plate IMRT Plans

Radiochromic film dose distribution analyses showed good concordance between the predicted and the measured radiation dose profiles across both X and Y axes for target doses of 2, 4, 8, and 16 Gy for all three tissue culture plates IMRT plans. However, at 24 Gy, measured radiation doses were lower than the predicted doses for all three IMRT plans, and this effect was likely due to the saturation of radiochromic films at this dose level. The 2D ion chamber array analyses showed good matching between the predicted and measured doses. Gamma index analysis was used to evaluate the percentage of agreement for pre-set dose and distance differences [13]. EPID analysis confirmed accurate delivery of all IMRT plans with gamma index pass rates of 99.5%, 93.1%, and 89.1% (pre-set tolerance of 3% for dose and 1% for distance differences) for 96-, 24-, and 6-well plate IMRT plans, respectively (Figure 3).

3.3. No Significant Differences in Cell Viability Signal, Clonogenic Potential, or γ-H2AX Foci Signal Levels between IMRT and Open-Field, Homogeneous Irradiations

For the A375 cell line, cell viability signal for 0, 2, 4, 8, 16, and 24 Gy with the IMRT plan were 2.08, 1.75, 1.42, 0.76, 0.55, and 0.55, respectively. With open-field, homogeneous irradiations, the corresponding values were 2.08, 1.65, 1.18, 0.65, 0.52, and 0.5. The differences between the techniques were not statistically significant (p = 0.82; Figure 4A). The corresponding values for the LN18 cell line were 2.01, 1.7, 1.39, 0.77, 0.27, 0.25, and 1.91; and 1.65, 1.33, 0.66, 0.35, and 0.37, respectively. Again, these differences did not reach statistical significance (p = 0.95; Figure 4B).
Figure 3. Cont.
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Figure 3. Dose distribution analyses of multiwell tissue culture plate IMRT plans. Screen shot images of radiochromic film (A); red line shows planned and green line shows predicted radiation doses across the X-axis), 2D ion chamber array (B); planned doses shown by orange line and measured doses by points), and EPID analyses (C); orange line shows predicted doses from the plan and green line shows measured doses).
The clonogenic assays showed that the A375 cell line colony numbers for 0, 2, 4, 8, 16, and 24 Gy were 208, 141, 74, 0, 0, and 0, respectively, and for the open-field homogeneous irradiations, they were 194, 143, 55, 0, 0, and 0, respectively. These differences in colony numbers between the two techniques did not reach statistical significance ($p = 0.92$; Figure 5A,B).

![Graph](image)

**Figure 5.** (A,B) Comparison of A375 colony numbers between 6-well tissue culture plate IMRT and open-field, homogeneous irradiations ($p = 0.92$). Results are reported as the average from three independent biological experiments. (C,D) Comparison of A375 γ-H2AX foci signal between 96-well tissue culture plate IMRT and open-field, homogeneous irradiations ($p = 0.75$). CellTag700 stain (in red) was used to stain nucleus and cytoplasm. γ-H2AX foci shown in green. Results are reported as the average from two independent biological experiments. Radiation doses are in gray (Gy). Error bars indicate standard deviation.
The γ-H2AX foci signal analyses showed a dose-dependent increase in signal intensity with increasing radiation dose. The mean γ-H2AX foci signal intensity for 0, 2, 4, 8, 16, and 24 Gy with IMRT plan was 4.74, 11.74, 18.68, 32.63, 60.51, and 88.40, respectively. The corresponding values for the open-field, homogeneous irradiations were 7.15, 16.33, 23.83, 41.35, 64.3, and 102.7. These differences were not statistically significant ($p = 0.75$; Figure 5C,D).

3.4. No Radiation Beam Inhomogeneity between and within Wells of Multicell Tissue Culture Plates

For MV irradiations (open-field and homogeneous), the mean radiation doses for the central wells were 2.00, 4.00, 8.09, 16.08, and 24.07 Gy, and for the edge wells, they were 2.01, 4.01, 8.02, 16.04, and 24.07 Gy. No consistent pattern of difference in cell viability signal between the central and the edge wells was observed for either A375 or LN18 cell lines or with either MV or KV irradiation. For 10 MV irradiations, the differences in cell viability signal between the central and the edge wells were not statistically significant ($p = 0.41$ for A375 and $p = 0.88$ for LN18). For 250 KV irradiation, no significant differences in cell viability signal between the central and the edge wells were observed ($p = 0.71$ for A375 and $p = 0.12$ for LN18; Figure 6A–D). The γ-H2AX foci signal analyses showed a homogeneous pattern of γ-H2AX foci for 8Gy KV irradiation (Figure 6E).

![Figure 6.](image)

Figure 6. (A–D) Comparison of cell viability signal between central and edge wells showing no significant differences. (A,B) MV irradiations, $p = 0.41$ for A375 cell line and $p = 0.88$ for LN18 cell line. (C,D) KV irradiations, $p = 0.71$ for A375 and $p = 0.12$ for LN18. (E) Homogeneous distribution of γ-H2AX foci (in green) for A375 cell line. CellTag700 stain (in red) was used to stain nucleus and cytoplasm. Superimposed images showing homogeneous γ-H2AX foci distribution related to cells (in yellow). Radiation doses are in Gy. Cell viability signal measured at 490 nm. Error bars indicate standard deviation.
4. Discussion

Recent advances in the discovery and development of new anticancer drugs provide an opportunity to enhance the efficacy of radiotherapy. To quickly and efficiently screen a large number of potential radiation–drug combinations, there is a need for a high-throughput in vitro radiobiology platform [14].

In the past, a modulated Cobalt-60 beam was used to vary radiation dose across a 96-well tissue culture plate for radiobiology experiments with a physical lead wedge used to modulate the beam [15]. However, Cobalt-60 teletherapy machines have largely been superseded by more modern and sophisticated linear accelerators that have the capability of producing multi-energy photon and electron beams. Multileaf collimators with varying functional capabilities have replaced traditional lead wedges. Modern linear accelerators can modulate radiation beams, and modulated radiation beams have already been used for radiobiology experiments [16]. A multiwell tissue culture plate based irradiation technique using homogeneous radiation dose distribution was developed for in vitro experiments [17]. Although this helps to investigate a range of drug doses in a single multiwell tissue culture plate, this has not eliminated the need for different plates for different radiation doses. It is possible to conduct cell viability experiments in 96-well plates with smooth dose gradients of up to 10.6 Gy with spatially distributed radiation [18]. A step- and-shoot radiation delivery technique was also used to deliver different radiation doses to a 96-well plate using treatment table movements along with clinical radiotherapy equipment [19] and there is potential for further improvement and refinements of this application.

Our high-throughput, in vitro radiobiology platform utilises widely available clinical radiotherapy facilities and IMRT, which has the ability to deliver different radiation doses to a single multiwell tissue culture plate. When cells are exposed to modulated radiation beams, due to the bystander effects of radiation, the proliferative capacity of cells is affected depending on experimental conditions [20,21]. To address this issue, in addition to the dosimetric verification of accurate dose delivery, we validated our platform by demonstrating bioequivalence between radiation doses delivered with tissue culture plate IMRT and conventional, open-field, homogeneous irradiations. Another concern with using IMRT for radiobiological experiments is the potential risk of intrafraction DNA repair due to the prolonged radiotherapy fraction treatment delivery times in IMRT [21–24], which potentially impacts the results of radiobiology experiments. This is an interesting area in radiobiology research that would need further exploration [25].

Our radiobiology platform has several advantages over previously described models. It allows testing of various radiation–drug dose combinations in a single multiwell tissue culture plate and the choice of three types of multiwell tissue culture plate allows our platform to be used for various in vitro radiobiology applications. As our platform uses widely available clinical radiotherapy facilities, it has the potential to enable more institutions to conduct radiobiology experiments. One limitation is that it is not possible to deliver the exact target radiation doses, although satisfactory mean target radiation doses were achieved. This is likely because of very steep radiation dose gradients given the dose range investigated. To address this limitation, one solution is to fit experimental results with linear regression modelling and derive results for target radiation doses. Another possible solution is to use the experimental result of each mean radiation dose as they were measured/calculated. Further technical advances in radiotherapy planning and equipment may help to overcome this limitation in the future.

Air cavities can influence radiation interactions with matter and its dose deposition [26]. When using multiwell tissue culture plates, because of the multiple air–tissue interfaces, there is concern about dose homogeneity between and within wells, which can affect the interpretation of the results and introduce uncertainties in radiobiology experiments. There was up to 4% underdosing at the air-fluid interface in multiwell tissue culture plate wells but this was not seen at the bottom of wells where cells were attached and grown [27] and our results confirm this observation, which may alleviate some of these concerns.
5. Conclusions

We developed a high-throughput in vitro radiobiology platform using IMRT and widely available clinical radiotherapy facilities. We dosimetrically verified the delivery of IMRT plans and biologically validated the platform by comparing it with conventional, open-field, homogeneous whole-plate irradiations. Such a radiobiology platform may help to make in vitro radiobiology experiments more efficient and cost effective.

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References

1. Delaney, G.; Jacob, S.; Featherstone, C.; Barton, M. The role of radiotherapy in cancer treatment. Cancer 2005, 104, 1129–1137. [CrossRef]
2. Begg, A.C.; Stewart, F.A.; Vens, C. Strategies to improve radiotherapy with targeted drugs. Nat. Rev. Cancer 2011, 11, 239–253. [CrossRef]
3. Formenti, S.C.; Demaria, S. Combining Radiotherapy and Cancer Immunotherapy: A Paradigm Shift. Jnci-J. Natl. Cancer Inst. 2013, 105, 256–265. [CrossRef] [PubMed]
4. Kahn, J.; Tofilon, P.J.; Camphausen, K. Preclinical models in radiation oncology. Radiat. Oncol. 2012, 7, 223. [CrossRef] [PubMed]
5. Coleman, C.N.; Higgins, G.S.; Brown, J.M.; Baumann, M.; Kirsch, D.G.; Willers, H.; Prasanna, P.G.S.; Dewhirst, M.W.; Bernhard, E.J.; Ahmed, M.M. Improving the Predictive Value of Preclinical Studies in Support of Radiotherapy Clinical Trials. Clin. Cancer Res. 2016, 22, 3138–3147. [CrossRef] [PubMed]
6. Stone, H.B.; Bernhard, E.J.; Coleman, C.N.; Deye, J.; Capala, J.; Mitchell, J.B.; Brown, J.M. Preclinical Data on Efficacy of 10 Drug-Radiation Combinations: Evaluations, Concerns, and Recommendations. Transl. Oncol. 2016, 9, 46–56. [CrossRef]
7. Singh, A.V.; Maharjan, R.S.; Kromer, C.; Laux, P.; Luch, A.; Vats, T.; Chandrasekar, V.; Dakua, S.P.; Park, B.-W. Advances in Smoking Related In Vitro Inhalation Toxicology: A Perspective Case of Challenges and Opportunities from Progresses in Lung-on-Chip Technologies. Chem. Res. Toxicol. 2021, 34, 1984–2002. [CrossRef]
8. Ford, E.; Deye, J. Current Instrumentation and Technologies in Modern Radiobiology Research-Opportunities and Challenges. Semin. Radiat. Oncol. 2016, 26, 349–355. [CrossRef]
9. Sheng, K. Artificial intelligence in radiotherapy: A technological review. Front. Med. 2020, 14, 431–449. [CrossRef]
10. Niroomand-Rad, A.; Blackwell, C.R.; Coursey, B.M.; Gall, K.P.; Galvin, J.M.; McLaughlin, W.L.; Meigooni, A.S.; Nath, R.; Rodgers, J.E.; Soares, C.G. Radiographic film dosimetry: Recommendations of AAPM Radiation Therapy Committee Task Group 55. Med. Phys. 1998, 25, 2093–2115. [CrossRef]
11. van Elmp, W.; McDermott, L.; Nijsten, S.; Wendling, M.; Lambin, P.; Mijnheer, B. A literature review of electronic portal imaging for radiotherapy dosimetry. Radiother. Oncol. 2008, 88, 289–309. [CrossRef] [PubMed]
12. Franken, N.A.; Rodermond, H.M.; Stap, J.; Haveman, J.; Van Bree, C. Clonogenic assay of cells in vitro. *Nat. Protoc.* **2006**, *1*, 2315–2319. [CrossRef] [PubMed]
13. Hussein, M.; Rowshanfarzad, P.; Ebert, M.A.; Nisbet, A.; Clark, C.H. A comparison of the gamma index analysis in various commercial IMRT/VMAT QA systems. *Radiother. Oncol. J. Eur. Soc. Ther. Radiol. Oncol.* **2013**, *109*, 370–376. [CrossRef] [PubMed]
14. Singh, A.V.; Gemmaiti, D.; Vats, T.; Singh, A.; Zamboni, P. High throughput array technologies: Expanding applications from clinics to applied research. *Front. Nanosci. Nanotechnol.* **2019**, *5*, 1–2. [CrossRef]
15. Cross, P.; Marshall, E.S.; Baguley, B.C.; Finlay, G.J.; Matthews, J.H.L.; Wilson, W.R. Proliferative assays for the assessment of radiosensitivity of tumor cell lines using 96-well microcultures. *Radiat. Oncol. Investig.* **1993**, *1*, 261–269. [CrossRef]
16. Regina, B.; Ross, D.; Lyn, O.; Rozelle, H.; Clive, B. A preliminary investigation of cell growth after irradiation using a modulated X-ray intensity pattern. *Phys. Med. Biol.* **2006**, *51*, 3639.
17. Tesei, A.; Sarnelli, A.; Arienti, C.; Menghi, E.; Medri, L.; Gabucci, E.; Pignatta, S.; Falconi, M.; Silvestrini, R.; Zoli, W.; et al. In vitro irradiation system for radiobiological experiments. *Radiat. Oncol.* **2013**, *8*, 257. [CrossRef]
18. Blockhuys, S.; Vanhoecke, B.; Paelinck, L.; Bracke, M.; Wagter, C.D. Development of in vitro models for investigating spatially fractionated irradiation: Physics and biological results. *Phys. Med. Biol.* **2009**, *54*, 1565. [CrossRef]
19. Tomic, N.; Gosselin, M.; Wan, J.F.; Saragovi, U.; Podgorsak, E.B.; Evans, M.; Devic, S. Verification of cell irradiation dose deposition using a radiochromic film. *Phys. Med. Biol.* **2007**, *52*, 3121–3131. [CrossRef]
20. Suchowerska, N.; Ebert, M.A.; Zhang, M.; Jackson, M. In vitro response of tumour cells to non-uniform irradiation. *Phys. Med. Biol.* **2005**, *50*, 3041–3051. [CrossRef]
21. Mackonis, E.C.; Suchowerska, N.; Zhang, M.; Ebert, M.; McKenzie, D.R.; Jackson, M. Cellular response to modulated radiation fields. *Phys. Med. Biol.* **2007**, *52*, 5469–5482. [CrossRef] [PubMed]
22. Moiseenko, V.; Duzenli, C.; Durand, R.E. In vitro study of cell survival following dynamic MLC intensity-modulated radiation therapy dose delivery. *Med. Phys.* **2007**, *34*, 1514–1520. [CrossRef] [PubMed]
23. Shibamoto, Y.; Ito, M.; Sugie, C.; Ogino, H.; Hara, M. Recovery from sublethal damage during intermittent exposures in cultured tumor cells: Implications for dose modification in radiosurgery and IMRT. *Int. J. Radiat. Oncol. Biol. Phys.* **2004**, *59*, 1484–1490. [CrossRef] [PubMed]
24. Morgan-Capner, P. Does the Time Required to Deliver IMRT Reduce Its Biological Effectiveness. *Int. J. Radiat. Oncol. Biol. Phys.* **2002**, *54*, 222. [CrossRef]
25. Blockhuys, S.; Vanhoecke, B.; De Wagter, C.; Bracke, M.; De Neve, W. From clinical observations of intensity-modulated radiotherapy to dedicated in vitro designs. *Mutat. Res. -Rev. Mutat. Res.* **2010**, *704*, 200–205. [CrossRef] [PubMed]
26. Klein, E.E.; Chin, L.M.; Rice, R.K.; Mijnheer, B.J. The influence of air cavities on interface doses for photon beams. *Int. J. Radiat. Oncol. Biol. Phys.* **1993**, *27*, 419–427. [CrossRef]
27. Sabater, S.; Berenguer, R.; Honrubia-Gomez, P.; Rivera, M.; Nunez, A.; Jimenez-Jimenez, E.; Martos, A.; Ramirez-Castillejo, C. How air influences radiation dose deposition in multiwell culture plates: A Monte Carlo simulation of radiation geometry. *J. Radiat. Res.* **2014**, *55*, 1009–1014. [CrossRef]