2D mapping of radiation dose and clonogenic survival for accurate assessment of \textit{in vitro} X-ray GRID irradiation effects

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Abstract
Spatially fractionated radiation therapy (SFRT or GRID) is an approach to deliver high local radiation doses in an ‘on-off’ pattern. To better appraise the radiobiological effects from GRID, a framework to link local radiation dose to clonogenic survival needs to be developed. A549 lung cancer cells were irradiated in T25 cm$^2$ flasks using 220 kV x-rays with an open field or through a tungsten GRID collimator with periodical 5 mm openings and 10 mm blockings. Delivered nominal doses were 2, 5, and 10 Gy. A novel approach for image segmentation was used to locate the centroid of surviving colonies in scanned images of the cell flasks. Gafchromic\textsuperscript{TM} film dosimetry (GFD) and FLUKA Monte Carlo (MC) simulations were employed to map the dose at each surviving colony centroid. Fitting the linear-quadratic (LQ) function to clonogenic survival data for open field irradiation, the expected survival level at a given dose level was calculated. The expected survival levels were then mapped together with the observed levels in the GRID-irradiated flasks. GFD and FLUKA MC gave similar dose distributions, with a mean peak-to-valley dose ratio of about 5. LQ-parameters for open field irradiation gave $\alpha = 0.24 \pm 0.02$ Gy$^{-1}$ and $\beta = 0.019 \pm 0.002$ Gy$^{-2}$. The mean relative percentage deviation between observed and predicted survival in the (peak; valley) dose regions was $(4.6; -1.0)$ %, $(26.6; -1.0)$ %, and $(129.8; -2.3)$ % for 2, 5 and 10 Gy, respectively. In conclusion, a framework for mapping of surviving colonies following GRID irradiation together with predicted survival levels from homogeneous irradiation was presented. For the given cell line, our findings indicate that GRID irradiation causes reduced survival in the peak regions compared to an open field configuration.

1. Introduction

In conventional radiation therapy (CRT), the treatment aim is to ensure that the whole tumor receives a homogeneous radiation dose. Spatially fractionated radiation therapy (SFRT) was originally introduced in 1909 by Köhler, where irradiation was performed through a perforated screen to deliver high radiation doses and, at the same time, to reduce extensive toxicity of skin and subcutaneous tissue (Laisse et al 2011). In SFRT, an ‘on-off’ field pattern is formed using an array of narrow parallel beamlets that are periodically spaced by a fixed gap, producing regions of low (valley) and high (peak) dose. Therefore, SFRT delivers a heterogenous dose distribution to the tumor, in contrast to CRT employing a homogeneous dose. SFRT has so far mainly been employed with a palliative treatment intent, where dose peaks higher than 10 Gy per fraction are commonly delivered (Billinga and Khan 2019, Yan et al 2020). Although SFRT delivery may be achieved using fixed collimators of dense metals or metal alloys such as Cerrobend, other solutions can also be utilized such as multileaf collimators (MLCs) (Ha et al 2006).
GRID is a two-dimensional (2D) treatment technique of SFRT, in which the beamlet size is typically in the range of 1.00–1.25 cm (Gholami et al 2017, Billena and Khan 2019). The dose is normally delivered as a single radiation fraction (10–20 Gy peak dose) in a systematic spatial pattern. GRID has been used both as monotherapy and in combination with external CRT and chemotherapy to enhance the local control of large and bulky tumors (Mohiuddin et al 1999, Huhn et al 2006, Peñagarícano et al 2010). Because of the small and discrete areas of irradiated healthy tissue by this method, higher doses may be tolerated than by homogeneous irradiation. Published clinical GRID therapy studies have shown beneficial palliative outcomes with reduced toxicity (Mohiuddin et al 1990, Mohiuddin et al 1996, Neuner et al 2012).

The primary aim of the current investigation was to develop appropriate tools that allow the mapping of both the dose deposition and the clonogenic survival following GRID irradiation in vitro. It is necessary to establish such a methodology to accurately appraise the radiobiological impact and to understand subsequent biological responses. Our proposed methodology consists principally of two parts: (1) film dosimetry to map the GRID dose distribution in the cell flasks and (2) mapping of surviving colonies. Importantly, dosimetry and colony scoring were done in the same spatial frame of reference. The dosimetry was done by Gafchromic™ films together with Monte Carlo (MC) simulations. Furthermore, clonogenic survival of A549 lung cancer cells was assessed following homogenous irradiation and fitted to the linear-quadratic (LQ) model. Applying the LQ model with the GRID dose distribution gives the predicted survival pattern if the cellular response was identical to that for homogenous irradiation. This enabled a comparison of the observed clonogenic survival following GRID irradiation to the survival predicted from open field irradiation. The developed framework is expected to facilitate high-quality studies of GRID effects and contribute to better insight into cellular response mechanisms following homogenous and heterogeneous dose deliveries.

2. Methods

2.1. Cell culture and clonogenic survival assays
Human non-small cell lung cancer (NSCLC) A549 cells (Giard et al 1973) were grown in a BioWhittaker 1:1 mix of Dulbecco’s Modified Eagle Medium (DMEM) and F12 with 15 mM Hepes and L-Glutamine, supplemented with 10% of fetal bovine serum (Biocrom), 1% penicillin/streptomycin (Lonza) and 200 units per litre insulin (Gibco). The cells were maintained in a fully humidified incubator providing 5% CO2 at 37 °C.

Before irradiation, the cells were seeded into T25 cm² flasks (Nunclon, Denmark) in 5 ml culture medium at density of 30,000 cells per flask to ensure appropriate colony scoring over a wide range of survival levels. To exclude artifacts from biological processes affected by cell density, the number of cells seeded was the same, and yielded equal plating efficiency (PE), for all experiments. This cell density was chosen as sufficiently high to be able to measure the effects of dose gradients in the penumbra regions of the grid, but sufficiently low for detection of individual colonies.

Cells were incubated overnight for 24 h prior to irradiation, allowing cells to adhere. After irradiation, the cell flasks were incubated for about 6 d before the colonies were fixated in 96% technical ethanol (Antibac, Norway) and stained with Methylene Blue (Sigma, USA). The cell flasks were then scanned in order to quantify the clonogenic survival using an in-house machine learning colony segmentation method (see section 2.4). Clonogenic survival was chosen as the endpoint to assess the effects of radiation, where survival was defined as the ability of a single cell to grow into a colony composed of more than 50 cells (Franken et al 2006).

2.2. Gafchromic™ film dosimetry (GFD)
GFD was used to measure the 2D dose distribution in the T25 cell culture flasks. The dosimetry experiment was conducted separately from the irradiation of the A549 cells. Gafchromic™ EBT3 films (lot No. 02122001) were handled and processed according to the recommended protocols specified for radiochromic film dosimetry in the report of AAPM Task Group 235 (Niroomand-Rad et al 2020).

Prior to irradiation, the EBT3 films were cut into smaller dimensions to fit inside T25 flasks (6.5 × 4.3 cm²), where the upper right corner was cut to mark the film orientation. Films were irradiated inside the flasks with open and GRID fields (see text below and figure 1). Using the same batch of EBT3 films, irradiation of dose calibration films was carried out prior to following film dose measurements and was conducted with delivered doses of \( D = 0, 0.1, 0.2, 0.5, 1, 2, 5 \) and 10 Gy. In order to reduce the statistical error, each calibration point consisted of eight irradiated film pieces (Devic et al 2006).

An Epson Perfection V850 Pro flatbed scanner (Epson, Japan) and its associated software EPSON Scan v3.9.3.3 were used to read all films 48 h after irradiation. To minimize film response uncertainty and variation due to film orientation dependence, nonuniformity and lack of reproducibility in the scanner response, all EBT3 films were scanned in the same orientation, positioned at a reproducible central location of the scan surface that was considered uniform. Each film was scanned four consecutive times. The digitized images were acquired in
transmission and RGB-positive mode with 16-bit depth per color channel and 300 dots per inch (dpi) spatial resolution, and without applying any image adjustments or color corrections. The images were saved in .tiff format.

All further image processing and analysis of the digitized images were done with MATLAB R2022a (MathWorks, Natick, MA, USA). The images were processed in three color channels (red, green and blue) and as grayscale image, where a predefined region of interest (ROI) of $4 \times 4$ mm$^2$ size (Gholizadeh Sendani et al 2018) was selected from the center of each calibration film to obtain the mean and standard deviation of the transmitted light intensity pixel value (PV). Utilizing an in-house made MATLAB script, the sampled mean PV was used to characterize the film response by the net optical density (netOD) with background correction (Devic et al 2016):

$$\text{netOD} = \log \left( \frac{\text{PV}_{\text{ctrl}} - \text{PV}_{\text{bckg}}}{\text{PV}_{\text{rad}} - \text{PV}_{\text{bckg}}} \right),$$

(1)

where $\text{PV}_{\text{ctrl}}$, $\text{PV}_{\text{rad}}$ and $\text{PV}_{\text{bckg}}$ are the averaged PV from images of control (unirradiated) EBT3 films, irradiated EBT3 films and ‘absolutely’ opaque sheets, respectively.

Furthermore, an appropriate analytical function was chosen to establish the calibration relationship between the netOD values and the known absorbed doses (Devic et al 2004):

$$D = a \cdot \text{netOD} + b \cdot \text{netOD}^n,$$

(2)

where $n$ initially was iteratively varied from 0.5 to 5.0 with a step of 0.5, because treating $n$ as a fitting parameter introduces a higher overall fit uncertainty while only negligibly improves the sum of residuals (Devic et al 2004). For every increment of $n$, calibration curves for the irradiated EBT3 films were constructed for each color channel (RGB and grayscale) of the scanned RGB images by fitting equation (2) with the method of least-squares. The goodness of the least-squares curve fitting was measured by $R^2$-squared and root-mean-square error (RMSE) for optimal color channel selection. The red channel was adopted for succeeding film dose measurements with $a_{\text{red}} = 6.3 \pm 0.1$, $b_{\text{red}} = 52.7 \pm 0.4$, $n_{\text{red}} = 3.0$, $R^2_{\text{red}} = 0.999$ and RMSE$_{\text{red}} = 0.0934$.

2.3. GRID and open irradiation

Either GRID (heterogenous) or open (homogeneous) field irradiation of A549 lung cancer cells and radiochromic EBT3 films was performed using a Pantak PMC 1000 x-ray unit (Pantak, USA). The tube was operated at 220 kV and 10 mA with 0.70 mm Cu and a 1.52 mm Al filtration. Using a Scanditronix-Wellhofer type FC65-G ionization chamber (IBA Dosimetries, Germany), the dose rate was measured to be $0.59 \pm 0.02$ Gy min$^{-1}$. The cell clonogenicity and dosimetry experiments were conducted at the same lateral position and source-surface distance (SSD) of 58.3 cm distance to the surface of the cell medium (see figure 1). GRID irradiation was done using a custom-made tungsten collimator with underneath placement of T25 cell culture flasks. Both the cells and radiochromic films were irradiated in T25 flasks, which were placed in the PMMA cell flask holder. For cell irradiation, the PMMA holder was placed on a preheated PMMA plate maintaining 37 °C in the medium of the flasks by circulating air.

For the A549 cell colonies, the delivered single doses were 0, 2, 5, and 10 Gy with 8 replicates per dose per field pattern. The reported doses are actual doses for the open field configuration, while they serve as nominal...
doses for the GRID dose distributions. For the EBT3 measurement films, single dose of 5 Gy was delivered to 16 film pieces per field pattern.

2.4. FLUKA Monte Carlo (MC)
MC simulations were performed using FLUKA code version 4–2.1 (Böhlen et al 2014, Battistoni et al 2015) and its graphical user interface Flair version 3.1–15 (Vlachoudis 2009). The simulations were used to model the experimental irradiation setup and to calculate the theoretical dose distribution in the region of the T25 flasks containing the EBT3 films (or analogously, the cell colonies). This served as a means to verify the GFD following open and GRID irradiation.

In the code, the focal spot of the x-ray unit along with the propagation of the primary x-ray photons to the EBT3 films contained in the T25 flasks was simulated, where the material geometry of the entire irradiation setup was modelled as described in section 2.3. The layer structure and corresponding elemental composition of the EBT3 films encased inside the flasks was also included in the modelling (Devic et al 2016). A customized routine was implemented in FLUKA that sampled a 220 kV x-ray energy spectrum attenuated through a 0.70 mm Cu and a 1.52 mm Al filter, which was generated by the computational toolkit SpekCalc (Poludniowski et al 2009). The spatial shape of the beam imitated the focal spot with a Gaussian lateral spread of 0.2 mm FWHM. Furthermore, the MC acquisition was performed by adopting default FLUKA physics settings (see PRECISION defaults in the FLUKA manual (Ferrari et al 2005)), where transport and production cut-off of photons and secondary electrons was set to 1 keV. In total, 2.0 × 10^7 primary x-ray photons were simulated.

Moreover, four scoring regions were defined for each EBT3 film piece within the flasks. Both dose deposition and fluence were obtained in the scoring regions by using the USRBIN option in FLUKA. Here, the quantities were scored in a spatial regular mesh of 732 × 507 × 1 uniformly sized bins enveloping the dimensions of the modelled EBT3 films of 6.5 × 4.3 × 0.0278 cm^3. The mesh resolution of the lateral scoring area was chosen to match the spatial resolution of the digitized EBT3 images for subsequent comparison.

2.5. Colony segmentation and localization
A novel machine learning approach using principal component–based watershed method for image segmentation was developed to identify individual colonies in a cell flask (Arous et al 2022). This automated algorithm was applied to scanned images of the cell culture flasks containing fixed and stained A549 cell colonies to locate the centroid of viable colonies. The images were processed with predefined parameters for the current cell line, where conglomerations composed of more than 50 cells were counted as a colony. Thereby, spatial 2D cell colony survival distributions were produced.

The image data were obtained from the flatbed scanner specified above for radiochromic dosimetry. The scanner provided RGB–images in transmission mode with 16-bit depth per color channel and 1200 dpi spatial resolution. For consistency, the cell flasks were positioned at the same location of the scan surface as the scanned EBT3 films. No prior filtering nor adjustments were performed on the captured images in conjunction with the scanning.

The colony segmentation procedure was previously developed and validated for sparsely seeded and unirradiated cells (Arous et al 2022). As the current work included densely seeded and irradiated cells, it was necessary to conduct a new, supervised calibration of the segmentation procedure. We compared the colony segmentation count to manual colony counts performed by three trained human observers. Each observer did not have access to the results of the others or the autosegmentation results when counting. Utilizing the A549 image dataset, each observer located one central coordinate point per colony representing the ground truth (GT) (see figure 3). This was done for ten image sections of 8 × 8 mm^2 size of A549 colonies following open field irradiation with nominal doses of D = 0, 2, 5 and 10 Gy. Hence, the GT count (NGT) for each observer was extracted together with the uncorrected autosegmentation count (Nseg) (see figure 3 and supplementary figure S1). From this, the autosegmentation method could be adjusted to the current application (see supplementary document). The factor used to calibrate the autosegmentation is given by:

\[
f(D) = \frac{N_{GT}(D)}{N_{seg}(D)}
\]

As seen in Supplementary figure S2, f(D) can be represented by a second order linear model with nominal dose D as independent variable.

2.6. Observation and prediction of local clonogenic survival
A direct spatial comparison of local colony survival distribution after GRID irradiation (observed survival) and the calculated survival for the measured GRID doses using parameters from a fit by the generalized LQ model (Fowler 1989) to data from open field irradiation (predicted survival) was performed (see figure 2).
Figure 2. Overview of the data acquisition and processing pipeline showing the main steps. Open or GRID irradiation with 220 kV x-rays was performed with a nominal dose $D$ of A549 colonies and Gafchromic$^\text{TM}$ EBT3 films in T25 cm$^2$ cell culture flasks. Subsequently, colony formation assays and radiochromic EBT3 films were scanned. An in-house developed image segmentation method (Arous et al 2022) was adopted in order to locate the centroid of each surviving colony. Gafchromic$^\text{TM}$ film dosimetry (GFD) was performed to measure the two-dimensional (2D) dose distribution in the cell flasks. FLUKA Monte Carlo (MC) simulation imitating the GRID irradiation experiments was done to verify the 2D GFD. From surviving fraction (SF) following open field irradiation, $\alpha$ and $\beta$ in the linear-quadratic (LQ) survival model was estimated by linear regression. Then, predicted survival levels in each pixel were mapped together with observed levels in the GRID-irradiated flasks.

From the cell colony segmentation algorithm, the centroids of the detected viable colonies defined the experimental cell survival maps in the culture flasks. In all irradiations, the surviving fraction (SF) was normalized to the PE of control flasks (i.e. sham treated and time-matched flasks):

$$\text{SF}(D)_{\Delta x} = \frac{N_{\text{count}}(D)_{\Delta x} \cdot f(D)}{N_{\text{seed}} \cdot \text{PE} \cdot f(0)},$$

where $\Delta x = 1$ mm is the band thickness longitudinally across the cell flask, $N_{\text{count}}(D)_{\Delta x}$ is the number of identified colony centroids within $\Delta x$ made by the segmentation algorithm for a local dose $D$, $f(D)$ is the corresponding correction factor for the segmentation count given by equation (3), $N_{\text{seed}} = 30\,000$ cells is the number of plated cells in all experiments. PE for the unirradiated A549 cells was estimated by the colony segmentation algorithm to be 21% (after calibration). The observed survival map was obtained by calculating the number of viable colony centroids within each $\Delta x$ for each cell flask and then taking the average for each $\Delta x$ over all cell flasks. From this, 95% confidence intervals (CIs) in experimental cell colony survival were obtained.

The dose map obtained from GFD was then used to estimate the dose at each surviving colony centroid. The predicted local SF was calculated from the LQ model fitted to data from open field irradiation:

$$\text{SF}(D(r)) = e^{-\alpha D(r) - \beta D^2(r)},$$

where $r$ is the position with coordinate $(x, y)$, $D(r)$ is the local dose, and $\alpha$ and $\beta$ are estimated by linear regression for A549 SF following open field irradiation. Similarly as for GRID-irradiated colonies, the predicted survival level in each pixel was mapped together with observed dose levels. Corresponding 95% CIs were also estimated for open and GRID field pattern.

3. Results

3.1. Dosimetry validation

FLUKA MC simulation imitating the open and striped GRID irradiation experiments was performed to verify the GFD. Utilizing the FLUKA code, the experimental setup was modelled and the dose distributions delivered to the EBT3 films contained in the cell culture flasks were calculated.

Having performed the GFD and scored the dose distributions with MC, central profiles were obtained across the 2D dose maps for validation. To scale these maps for comparison, they were normalized with respect to respective averaged dose profiles for open irradiation (see figure 4). When irradiating the films with a nominal 5 Gy dose, the films exposed to an open field exhibit a 5 Gy response on average, whereas using the striped GRID, the average valley dose was found to be 0.9 Gy and the average peak dose to be 4.1 Gy (see table 1). Hence with GRID irradiation, about 18% of the dose deposition is lost in the peak regions when compared to open field irradiation. The GFD and MC simulations gave highly similar dose distributions, with a mean peak-to-valley dose ratio (PVDR) of 4.7 (4.5, 4.9) and 5.2 estimated from the EBT3 dosimetry and FLUKA MC simulations, respectively.
3.2. Survival comparison after open and GRID irradiation

After calibrating the colony segmentation procedure, the LQ model was fitted to clonogenic survival of A549 cells for open field irradiation by linear regression (see figure 5), giving parameter values of $\alpha = 0.24 \pm 0.02$

![Figure 3](image_url)

**Figure 3.** (a) Example image from a T25 cm$^2$ cell culture flask containing A549 colonies after open field irradiation with 2 Gy. (b) Zoomed-in section showing $8 \times 8$ mm$^2$ region of interest (ROI), where delineations suggested by the colony segmentation algorithm is presented in black for 2 Gy dose of open irradiation. Ground truth (GT) marks established by three observers are presented as red, green and blue asterisks, respectively.

![Figure 4](image_url)

**Figure 4.** (a) 2D dose distribution for GRID irradiation measured by EBT3 GFD. (b) 2D dose distribution scored with FLUKA MC simulations. (c) Normalized dose profiles from GFD of open and GRID irradiation (red and blue lines, respectively, with correspondingly colored bands indicating 95% CI) together with corresponding profiles from MC simulations (black and grey lines, respectively, with correspondingly colored dots indicating MC dose data). Dose profiles from MC simulations are smoothed using moving averages.

| Open       | Valley dose (Gy) (95% CI) | Peak dose (Gy) (95% CI) |
|------------|--------------------------|------------------------|
| 5.0 (4.8, 5.1) | 0.9 (0.8, 0.9)           | 4.1 (3.9, 4.2)         |

Table 1. Dose estimates from EBT3 films following open and GRID irradiation together with 95% CIs estimated from the Gafchromic™ film dosimetry (GFD).
$\gamma_{-1}$ and $\beta = 0.019 \pm 0.002 \text{Gy}^{-2}$. Both parameters were statistically significant ($p$-value $\leq 0.001$). The goodness-of-fit measures were $R^2 = 0.991$ and $\text{RMSE} = 0.164$.

Having established the values of $\alpha$ and $\beta$, the LQ model from equation (5) was deployed on the measured EBT3 dose distributions to produce 2D maps of the predicted SF (see figure 6(a)). The experimental SF was found using the calibrated image segmentation method, in which the centroid of each surviving cell colony was located (see figure 6(b)). Equation (4) was then employed to map the average observed SF profiles in the cell flask to compare with the predicted profiles as shown in figure 6(c) for nominal dose of 5 Gy. Qualitatively, mapping the SF longitudinally along the cell flask gave a pattern resembling the GRID collimator outline, where the average observed and predicted survival profiles also gave similar patterns. However, significantly lower observed survival was found in the peak regions compared to predicted survival for all GRID deliveries. No significant discrepancies were seen in the valley regions for all nominal doses, although a slightly lower survival
of valley cells compared to the predicted LQ-response was seen for 2 Gy of nominal dose (see figure 6(c), supplementary figures S3(c) and S4(c)). Quantitatively, the mean relative percentage deviation (RPD) between the observed and predicted survival in valley and peak dose regions are shown in table 2. Striped GRID irradiation with 10 Gy nominal dose caused the greatest difference from the prediction model based on homogenous irradiation. For open irradiation, although mean RPD increased slightly with dose, no significant discrepancies between observed and predicted survival were evident (RPD < 8.5%; see figure 6(c), supplementary figures S3(c) and S4(c)).

### 4. Discussion

In the current study, by delivering a highly non-uniform dose distribution using a specially constructed grid collimator, the clonogenic survival of A549 cells following GRID irradiation was measured and compared to the survival predicted from open field irradiation data. GFD and automated colony counting was used for pixel-by-pixel mapping of radiation dose and cell colonies, respectively.

The GRID dose distribution obtained from GFD was validated by MC simulations. The scored dose deposition maps from MC confirmed the finding from the film dosimetry of a lower peak dose relative to open field irradiation with the same nominal dose. Also, the valley dose was larger than 0 Gy in the shielded areas (see figure 4). The 18% drop in dose from open field to peak GRID region is due to the fact that the collimator will effectively attenuate diverging and scattered x-rays. The observed dose difference of 18% is however specific for the 5 mm striped fields. Bigger grid openings will yield less difference between peak dose and open dose deposition. Moreover, our detection of a low valley dose from scattered radiation in the collimated regions is in line with other in vitro studies (Mackonis et al 2007, Asur et al 2012, Peng et al 2017), where the valley dose is primarily dependent on the beam energy and modulation. For instance, Mackonis et al (2007) measured approximately 6% valley dose relative to the prescribed dose for 6 MV striped intensity-modulated radiation therapy (IMRT) fields created by 0.75 cm openings and 2.25 cm MLC shielding. Comparable to our collimated x-ray beam, Peng et al (2017) generated a striped GRID pattern of periodicity of 5.0 mm spacing, which were characterized dosimetrically using radiochromic EBT3 film, where valley regions showed approximately 20% of prescribed dose. Other studies have reported an out-of-field dose <10% of the in-field dose (McGarry et al 2012, Trainor et al 2012).

Several publications have stated that steep dose gradients associated with GRID IMRT (generally 6 MV x-ray beams) overall suggest significantly greater decrease in survival than expected in the collimated valley regions and unexpected increase in survival in the open peak regions for malignant melanoma (MM576), human prostate cancer (DU-145) and normal human fibroblast (AGO-1552) cells (Mackonis et al 2007, Butterworth et al 2011, McGarry et al 2012, Trainor et al 2012). Asur et al (2012) also reported unexpectedly high cell kill in the valleys for confluent murine mammary carcinoma (SCK) and head and neck sarcoma (SCCVII) cells following exposure to a single dose of 10 Gy using circular GRID opening of 12 mm diameter and a center-to-center distance of 18 mm (nearly 50:50 valley and peak exposure). In conclusion, these in vitro investigations of clonogenic survival demonstrated higher survival in the peaks and lower survival in the valleys.

We observed a significantly lower clonogenic survival of cells located in the peak regions compared to the predicted LQ-response, in contrast to previously published studies (Mackonis et al 2007, Butterworth et al 2011, McGarry et al 2012, Trainor et al 2012, Asur et al 2015). The survival difference increased with increasing peak dose. We also observed a slightly lower survival level compared to the predictions in the gradient zones (figure 6(c), supplementary figures S3(c) and S4(c)), although our data does not allow any firm conclusion. Such a difference could be attributed to bystander cell killing, mediated either by cell-cell contact via gap-junction communication or through secretion of soluble factors, which is absent from the classical LQ model formalism.

| Mean RPD | Open | GRID (peak; valley) |
|----------|------|---------------------|
| Dose (Gy) | %     | %                   |
| 2.0      | 2.3%  | (4.6; 3.1)%         |
| 5.0      | 4.2%  | (26.6; −1.0)%       |
| 10.0     | 8.4%  | (129.8; −2.3)%      |
Refinement of the classical LQ model with a bystander term have been reported (Peng et al. 2017, Peng et al. 2018). These modified LQ models are based on the assumption that the generation of bystander signaling is linear in local dose. Therefore, this formulation increases the radiosensitivity contribution of the parameter α, which might explain the linearly increasing discrepancies in peak dose regions between the observed and predicted cell survival levels (see table 2). Ultimately, it can be seen that the standard LQ model is not supported to be used to predict survival response for GRID fields, which will be further assessed in future GRID studies.

5. Conclusion

A framework for detailed mapping of surviving cancer cell colonies following GRID irradiation together with predicted survival levels based on data from open field irradiation has been presented. Our findings, confirmed by MC simulations, indicate that GRID irradiation causes lower doses in the peak regions than expected from the nominal dose. In addition, the observed cell survival was lower than what is predicted from the actual doses in peak regions. The current developed methodology will aid future radiobiology investigations of GRID effects and cellular mechanisms causing the altered responses.

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Conflict of interest

The authors have no conflict of interest to report. The authors alone are responsible for the content and writing of this article.

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