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Dependence of LET on material and its impact on current RBE model

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Abstract

Biological uncertainty remains one of the main sources of uncertainties in proton therapy, and is encapsulated in a scalar quantity known as relative biological effective (RBE). It is currently recognised that a constant RBE of 1.1 is not consistent with radiobiological experiment and may lead to sub-optimal exploitation of the benefits of proton therapy. To overcome this problem, several RBE models have been developed, and in most of these models, there is a dependence of RBE on dose-averaged linear energy transfer (LET), LET_D. In this work, we show that the LET_D estimation in these models during the data-fitting (or parameter estimation) phase could be subjected to a huge uncertainty due to not taking into account cellular materials during simulation, and this uncertainty can propagate down to the resulting RBE models. The dosimetric impact of this LET_D uncertainty is then evaluated on a simple clinical spread out Bragg peak (SOBP) and a prostate example. Our simulation shows that LET_D uncertainty due to the use of water as cellular material is non-negligible under low $(\alpha/\beta)_X$ and low dose (2 Gy), and can be neglected otherwise. Thus, this study indicates that further dose and range margins may be required for low $(\alpha/\beta)_X$ target under low dose. This is due to greater uncertainties in RBE model associated with incomplete knowledge of cellular composition for LET_D computation.

1. Introduction

One advantage of proton therapy lies in the high dose conformity arising from the Bragg peak (Newhauser and Zhang 2015) which could potentially lead to increased normal tissue sparing and an increased therapeutic index. However, this advantage cannot be fully exploited without eliminating the uncertainty problems (Paganetti 2013) in proton therapy. The biological uncertainty is encapsulated by a single scalar quantity known as *relative biological effectiveness (RBE)*, which is defined as the ratio of the reference photon dose to the proton dose to induce the same biological effect. Traditionally, most proton therapy Centres assumed RBE to have a constant value of 1.1 at all spatial positions, despite experimental and simulation evidences indicating otherwise (Paganetti 2014, 2018, Mohan *et al* 2017, Luhr *et al* 2018). Variable RBE models are currently being developed to optimize proton therapy clinical outcomes and to better understand clinical (and experimental) results originating from proton therapy. Thus development of well-validated variable RBE model is highly important in proton therapy.

RBE is a complicated quantity that depends on the type of particle, the type of cell or tissue, the delivered dose and the biological endpoint. The nature or *quality* of the particle can be represented by a dosimetric quantity known as dose-averaged LET (LET_D). This is motivated by experimental studies in Folkard *et al* (1996), Coutrakon *et al* (1997) and Wouters *et al* (1997) which suggest a strong and positive correlation between LET_D and RBE. Current RBE models can be classified as LET-dependent (Hawkins 1998, Wilkens and Oelfke 2004, Kase *et al* 2007, Carabe *et al* 2012, Wedenberg *et al* 2013, Jones 2015, McNamara *et al* 2015) or LET-independent (Semenenko and Stewart 2006, Frese *et al* 2012, Stewart *et al* 2015). Most of the LET-dependent models (Wilkens and Oelfke 2004, Carabe *et al* 2012, Wedenberg *et al* 2013, Jones 2015, McNamara *et al* 2015) are based on

linear-quadratic (LQ) models (Joiner and dan der Kogel 2009) which are widely used in traditional radiotherapy and can be easily implemented due to the existence of a single parametric form. The exact parametric form is derived from performing regression analysis on radiobiological data for various biological endpoints (such as clonogenic cell survival, double-strand breaks or chromosome aberrations) and cell lines.

The most comprehensive radiobiological data sets were reported by Paganetti (2014). However, most of the given LET_D in Paganetti (2014) contains inherent uncertainties due to *different definition* of LET and the use of water as *surrogate material* for LET_D computation. Paganetti *et al* extracted the LET values from various literatures but these LETs were defined differently in them (not all were defined as LET_D) and no distinctions were made in Paganetti (2014). There were three main definitions that were used—(1) *pure* − LET, (2) Track-averaged LET (LET_t) and (3) LET_D. The first definition is used often in mono-energetic proton experiment and the value is extracted from the *stopping power* of proton in water. This definition fails to take into account that the proton beam is composed of many particles which are slowed down by various degrees stochastically in the medium leading to energy straggling. Definition two and three are macroscopic quantities representing *mean LET* for a proton beam with an energy distribution at any spatial location. It is defined as

$$\mathrm{LET}_t(\vec{r}) = \frac{\int \Phi(E,\vec{r})\mathrm{LET}(E)dE}{\int \Phi(E,\vec{r})}, \tag{1}$$

and

$$\mathrm{LET}_D(\vec{r}) = \frac{\int D(E,\vec{r})\mathrm{LET}(E)dE}{\int D(E,\vec{r})}, \tag{2}$$

where $\Phi(E,\vec{r})$ is defined as the fluence of proton with energy E at location $\vec{r}$ and $D(E,\vec{r})$ is the dose deposited by proton with energy E at location $\vec{r}$. It is shown in Guan *et al* (2015) that equations (1) and (2) are generally different especially when using small step size of less than 100 μm, and at the plateau region of the depth-dose curve. Despite the differences in the LET definitions, $\mathrm{LET} \approx \mathrm{LET}_d \approx \mathrm{LET}_t$ for high energy proton and very thin material. This is because under these two conditions, energy straggling effect on the primary proton is minimal and $\Phi(E) \approx \delta(E - E_0)$, where $\delta(E)$ is the Dirac delta function and E_0 is the energy of primary proton. Inputting this expression into both equations (1) and (2) will result in an integration result of just $\mathrm{LET}(E_0)$ which coincides with the pure- LET definition. To ensure consistency in the data sets in Paganetti (2014), LET_D values were calculated in this work for all the data sets which uses different definitions of LETs. No calculation is carried out for data points which reported LET_D.

All the LQ-based models are currently fitted using the assumption that the cell or tissue in experiment are made up of solely water and the LET_D are determined under such assumption. This assumption could lead to an under-estimation of the actual LET_D values in experiment and result in an inaccurate RBE model. The impact of types of RBE models on resulting dose distributions had been analysed in detail by Giovannini *et al* (2016) but the impact of types of cellular materials has not been reported and will be analysed in depth in this work. In this work, we used realistic cytoplasm composition determined in Incerti *et al* (2009) and Byrne *et al* (2013) to re-calculate all the LET_D values from the radiobiological data in Paganetti (2014). This involves extensive literature review to understand the experimental set-up to calculate the LET_D in the most accurate manner. The aim of this work is to re-derive the LQ-based RBE model by using the corrected LET_D values and compared to the previous models. RBE models are then compared using clinical examples to evaluate the potential dosimetric errors when the LET_D is not calculated accurately in experiments. Such comprehensive studies on the dependence of cellular materials on LET_D and its impact on RBE model has not been reported before and we believe this work represents a significant step towards better and more robust RBE modelling.

2. Methods and materials

2.1. Overview of phenomenological RBE model

This section presents an overview of the RBE model based on the LQ framework. The RBE of protons can be deduced by equating the biological effect of the proton beam with the reference radiation using the LQ equation to yield

$$\mathrm{RBE}\left[D_p, \left(\frac{\alpha}{\beta}\right)_X, \mathrm{LET}_D\right] = \frac{1}{2D_p}\left(\sqrt{\left(\frac{\alpha}{\beta}\right)_X^2 + 4D_p\left(\frac{\alpha}{\beta}\right)_X \mathrm{RBE}_{max} + 4\mathrm{RBE}_{min}^2 D_p^2} - \left(\frac{\alpha}{\beta}\right)_X\right). \tag{3}$$

D_p is defined as the dose delivered by the proton, $(\alpha/\beta)_X$ is defined as the α and β parameters in the LQ model for the reference radiation (x-ray photon). The RBE_{max} and RBE_{min} represents the RBE values at limiting dose of $D \to 0$ Gy and $D \to \infty$ Gy respectively and are defined as

$$\mathrm{RBE}_{max} = \frac{\alpha[\mathrm{LET}_D]}{\alpha_X}, \tag{4}$$

$$\mathrm{RBE}_{min} = \sqrt{\frac{\beta[\mathrm{LET}_D]}{\beta_X}}. \tag{5}$$

The $\alpha[\mathrm{LET}_D]$ and $\beta[\mathrm{LET}_D]$ represents the tissue response of proton radiation in the LQ formalism and is a function of the energy or LET_D of proton beam. The RBE model in equation (1) can be seen as a *functional* of RBE_{max} and RBE_{min} and different parameterisation of these two functions were adopted by different authors. There are three main parameterisations. The Wedenberg *et al* model in Wedenberg *et al* (2013) assumed

$$\mathrm{RBE}_{min} = 1, \qquad \mathrm{RBE}_{max} = p_0 + p_1 \frac{\mathrm{LET}_D}{(\alpha/\beta)_X}. \tag{6}$$

The Carabe *et al* model in Carabe *et al* (2012) assumed

$$\mathrm{RBE}_{min} = p_0 + p_1 \frac{\mathrm{LET}_D}{(\alpha/\beta)_X}, \qquad \mathrm{RBE}_{max} = p_2 + p_3 \frac{\mathrm{LET}_D}{(\alpha/\beta)_X}. \tag{7}$$

Lastly, the Jones *et al* model in Jones (2015) assumed

$$\mathrm{RBE}_{min} = p_0 + p_1 \sqrt{\left(\frac{\alpha}{\beta}\right)_X} \mathrm{LET}_D, \qquad \mathrm{RBE}_{max} = p_2 + p_3 \frac{\mathrm{LET}_D}{(\alpha/\beta)_X}. \tag{8}$$

The p_i in equations (4)–(6) are the fitting parameters and are deduced by performing regression analysis on experimental data with reported values on $\alpha[\mathrm{LET}_D]$, $\beta[\mathrm{LET}_D]$, $(\alpha/\beta)_X$ and LET_D. Due to the paucity of data in the entire $\left(D_p, (\alpha/\beta)_X, \mathrm{LET}_D\right)$ hyperspace, it is difficult to decide on the most accurate RBE models. Thus, all of the three models are fitted to the revised data in this work to examine the influence of LET_D on RBE model.

All the calculations of dose and LET_D are accomplished using Monte Carlo simulation with GEANT4 (Agostinelli *et al* 2003, 2006, Allison *et al* 2016). The phase space information of the proton beam used to generate the spread out Bragg peak (SOBP) and pristine Bragg peak in the simulation follows from the spot scanning proton therapy system from Hitachi Ltd Tan *et al* (2019). The LET_D distribution in our simulation agrees with the simulation data in Paganetti (2014) where the LET_D ranges from 2.0 to 3.0 keV $\mu\mathrm{m}^{-1}$ at the center of SOBP and increases sharply at the *distal fall-off* (below 20 keV $\mu\mathrm{m}^{-1}$). We use the FTFP_BERT physics model with standard EM model which is the recommended physical model for clinical proton beam below 5 GeV Guan *et al* (2015). The maximum step size is chosen to be 0.5 mm to avoid unphysical artefacts in the LET_D distribution (Guan *et al* 2015) and the length cut-off of 0.7 mm is chosen to ensure a smooth LET_D distribution. However, a smaller cut-off length and step size is used in calculating the dose distribution for accurate distribution. Lastly, the scoring of dose and LET_D quantities are done in 1 mm voxel.

2.2. LET_D dependence on biological materials

In the actual calculation of RBE with CT data, the material in each voxel is defined by the HU value which is converted to *stopping power* for dose calculation in commercial treatment planning system (TPS). Similarly, actual material should also be factored into the calculation of LET_D during radiobiological experiment. All the LET_D data in Paganetti (2014) are calculated with water as the surrogate material. Thus, in this section, we will examine the dependence of LET_D on different cellular materials, which should be the relevant material of consideration during *in vitro* or *in vivo* irradiation. The elemental composition of cytoplasm and nucleus from Incerti *et al* (2009) and Byrne *et al* (2013) are shown in table 1. The water composition is included in the table for comparison. The difference in cellular composition in table 1 are due to different methods of determining the compositions. The cellular composition in Incerti *et al* (2009) is obtained by ion beam analysis techniques with Rutherford backscattering (RBS) and proton-induced x-ray emission (PIXE) of a human keratinocyte cell line, whereas the composition in Byrne *et al* (2013) is obtained from estimation of the data published in ICRU report 44 and soft tissue data in White *et al* (1987).

2.3. LET_D computation in different irradiation configurations

Accurate determination of LET_D for each data set requires a close examination of the experimental set-up and biological protocol to set up the most realistic Monte Carlo simulation. Geometry setup of the simulation is divided into 4 scenarios as shown in figure 1. Configuration P2 is used if the irradiation is carried out with a monoenergetic proton beam through a *monolayer* cell culture and the proton beam is sufficiently energetic to transverse the entire cell. In this case, the cell thickness is assumed to be 10 μm and the LET_D is calculated within this small volume. Configuration P1 is used when the entire bragg peak fall within the cell sample either due to low energy proton or the use of a cell suspension (not a monolayer cell) in a flask. Configuration S2 is

Table 1. A table showing the elemental mass composition of cellular materials—cytoplasm and nucleus, from Incerti *et al* (2009) and Byrne *et al* (2013). The composition of water is included for comparison.

	Elemental mass composition(%)				
Element	Cytoplasm Incerti *et al* (2009)	Cytoplasm Byrne *et al* (2013)	Nucleus Incerti *et al* (2009)	Nucleus Byrne *et al* (2013)	Water
Carbon	13.01	29.88	12.25	9.00	0
Hydrogen	21.86	10.55	21.77	10.60	11.11
Oxygen	62.34	56.30	62.35	74.20	88.89
Nitrogen	1.29	2.51	2.13	3.2	0
Sodium	0	0.11	0.04	0	0
Magnesium	0	0	0.03	0	0
Silicon	0.06	0	0.01	0	0
Phosphorus	0.48	0	0.60	0	0
Sulphur	0.11	0.24	0.12	0.40	0
Chlorine	0.29	0.16	0.10	0	0
Potassium	0.57	0.21	0.60	0	0
Total	100	100	100	100	100

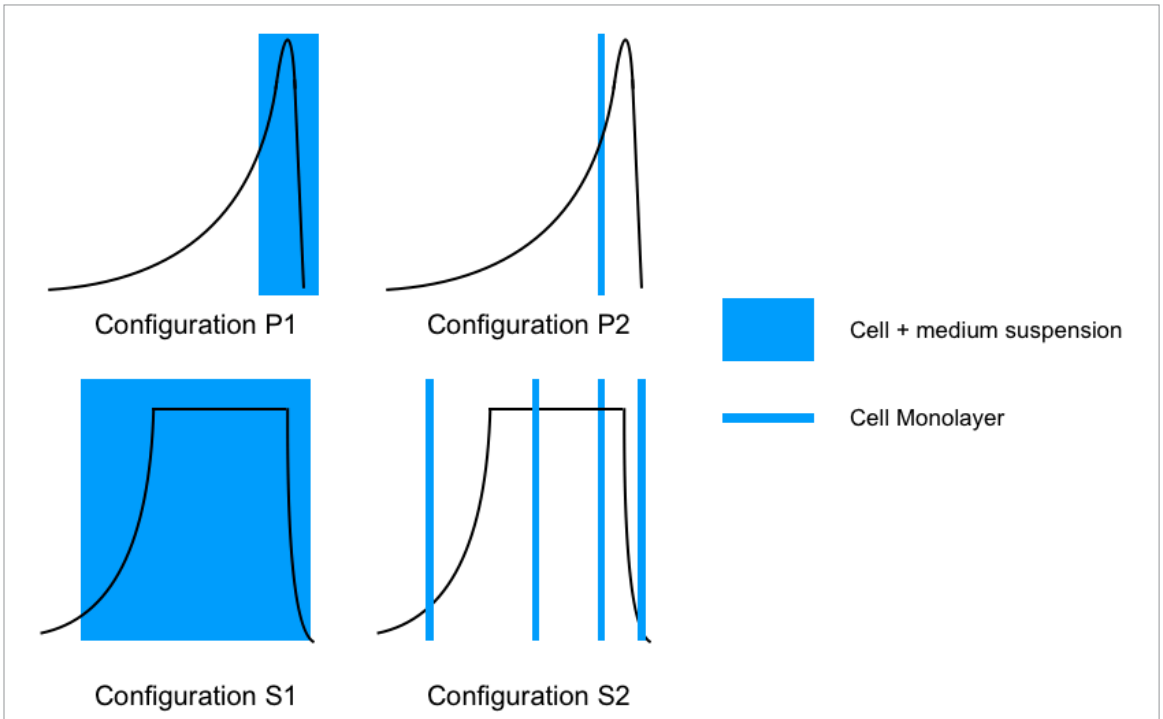

Figure 1. This figure shows the classification of the configurations of the irradiation set-up. (top left) Mono-energetic proton with bragg peak lying entirely within the cells. (top right) Mono-energetic proton with sufficient energy to exit the cells. (bottom left) Irradiation using SOBP with the cells lying throughout the proton beam. (bottom right) Irradiation using SOBP with thin layers of cells lying at specific location of SOBP.

used when a *monolayer* cell culture is irradiated with an energy-modulated proton beam or SOBP. The cells are often irradiated either at the plateau or the SOBP region of the depth-dose in experiments. The thickness of the monolayer cells is assumed to be 10 μm unless otherwise stated in the journal. Configuration S1 is used for experimental technique developed by Skarsgard *et al* (1982) where the cell and medium are mixed with gelatin in a long tube which solidify under low temperature. This tube is then irradiated with the SOBP lying entirely within it which is subsequently sliced at different spatial interval to perform analysis and assay. Thus under such configuration, the entire irradiated volume will assume the cellular composition and only the data sets from Wouters *et al* 1997, 2015 and Raju *et al* 1978a, 1978b employ this technique. One of the four scenarios is assigned to each data point based on the information given in the journal and is indicated in the sixth column of the data table A1, A2, A3 and A4 in appendix. The two main information we looked out for in literature for assignment are the type of proton beams (monoenergetic or modulated) and design of cell irradiation target.

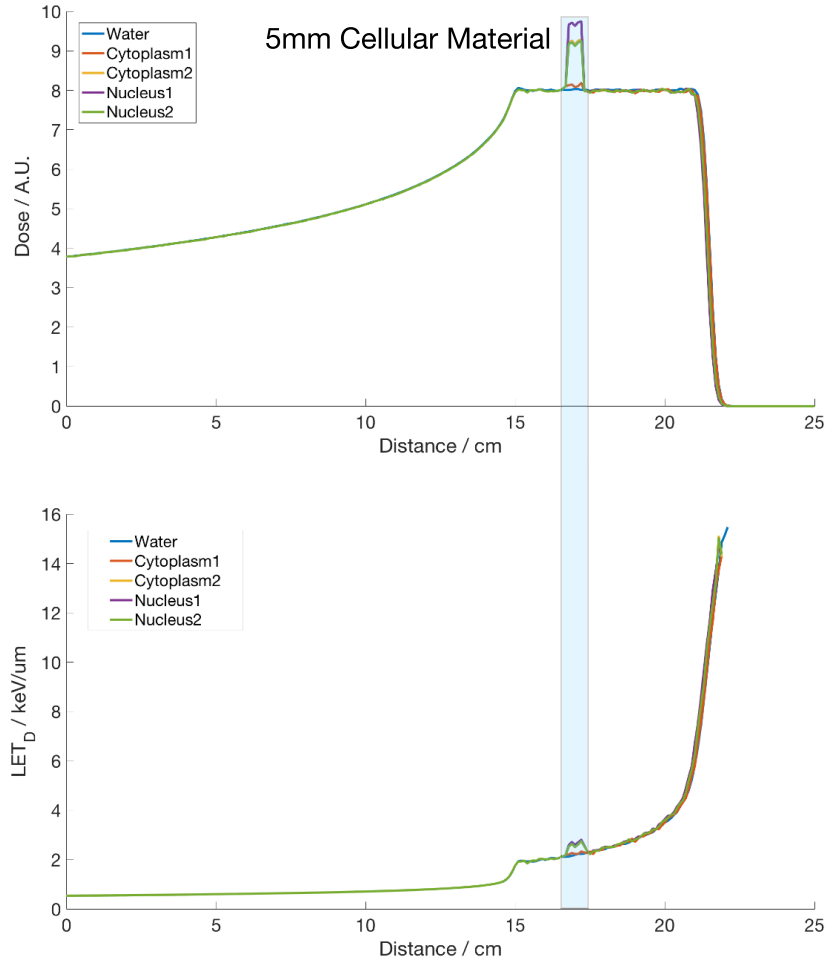

Figure 2. This figure shows the effect of cellular materials stated in table 1 on the dose (top) and LET_D (bottom) values of a proton SOBP. The cellular materials of 5 mm thickness are placed at 17 cm depth indicated by the blue band in the figure. Non-negligible increase in dose and LET_D values are observed at the positions where the cellular materials are located.

2.4. Omission of data

Not all the data points from the original compilation by Paganetti *et al* were used and the removed data points were absent in the data table in appendix. The removal of data points was due to the following constraints:

(i) Only data points with $(\alpha/\beta)_X < 30$ Gy and $\mathrm{LET}_D < 20\,\mathrm{keV}\,\mu\mathrm{m}^{-1}$ were used for analysis in later section due to clinical relevance in proton therapy. This criteria was also employed in the work by McNamara *et al* (2015). No calculation of LET_D was performed for monoenergetic proton experimental studies involving *pure* $-$ LET $> 50\,\mathrm{keV}\,\mu\mathrm{m}^{-1}$ (these data points will not appear in appendix).

(ii) Data points obtained from *microbeam experiments* with *several microns* beam spots were removed due to recent evidence of differential response of tumors irradiated with microbeam and clinical beam (Grotzer *et al* 2015, Girst *et al* 2016, Prezado *et al* 2017, Friedrich *et al* 2018). This excludes data from Folkard *et al* (1996) and Schettino *et al* (2001).

(iii) Some data points are obtained from experiment involving high dose rate which are clinically unachievable and irrelevant. These data points were removed and include (Doria *et al* 1996).

2.5. Evaluation of dose difference

The impact of the RBE models derived from different cellular materials (water, cytoplasm-1, cytoplasm-2) are evaluated on the dose distributions (with RBE weighting) of two different scenarios. The first scenario is a clinical SOBP and the second is an actual clinical prostate case.

For the clinical SOBP, the dosimetric difference between RBE models of different materials are evaluated based on mean dose (D_{mean}) and maximum dose (D_{max}) at the SOBP region. For the second scenario with an actual clinical case, the prostate is irradiated with two opposing lateral proton fields. The spot weights are

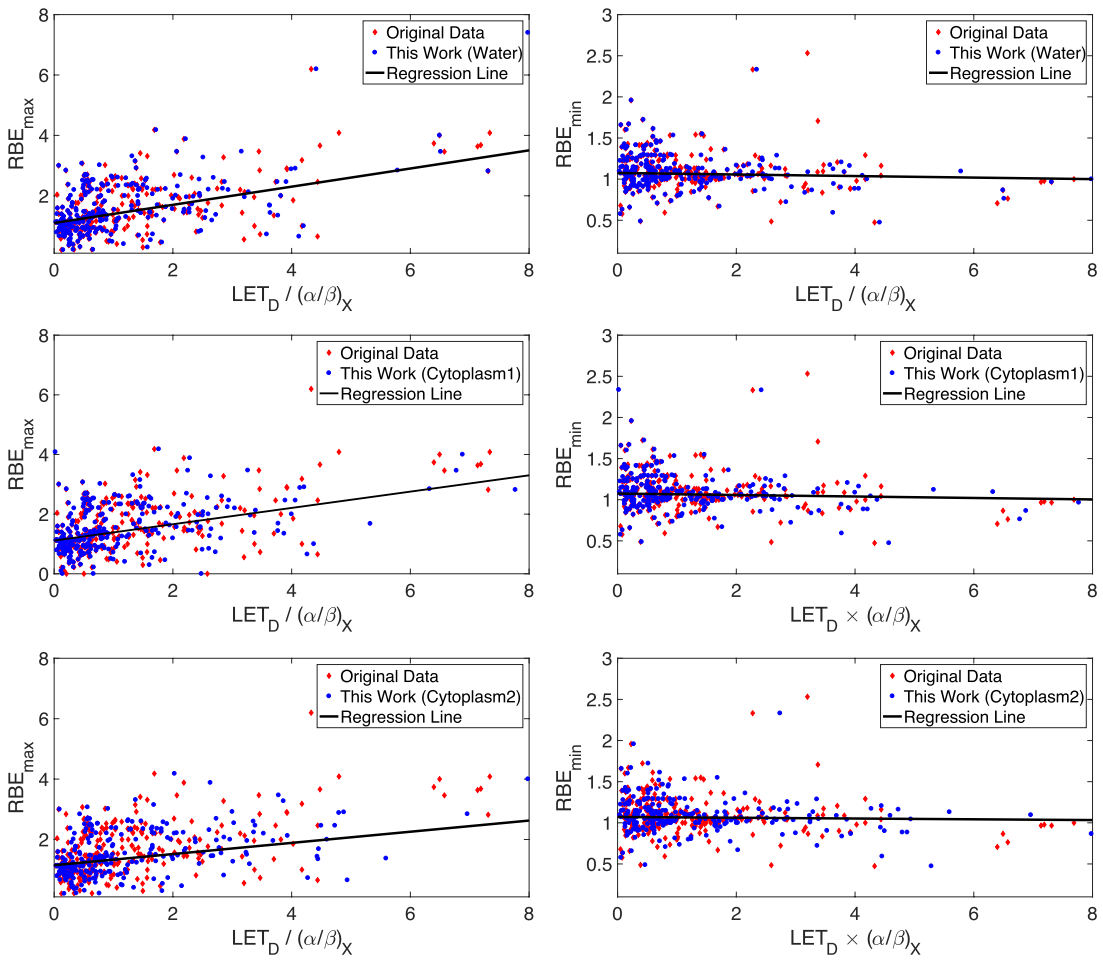

Figure 3. All the blue solid circles in the 6 plots represent the data points from Paganetti (2014) and the red solid diamonds represent simulation data from this work. The regression lines are fitted to our simulation data. (top left) RBE_{max} against $LET_D/(\alpha/\beta)_X$ with red diamond representing water data. (middle left) RBE_{max} against $LET_D/(\alpha/\beta)_X$ with red diamond representing cytoplasm from Incerti *et al* (2009). (bottom left) RBE_{max} against $LET_D/(\alpha/\beta)_X$ with red diamond representing cytoplasm from Byrne *et al* (2013). (top right) RBE_{min} against $LET_D/(\alpha/\beta)_X$ with red diamond representing water data. (middle right) RBE_{min} against $LET_D/(\alpha/\beta)_X$ with red diamond representing cytoplasm from Incerti *et al* (2009). (middle left) RBE_{min} against $LET_D/(\alpha/\beta)_X$ with red diamond representing cytoplasm from Byrne *et al* (2013).

determined from optimization in our Varian eclipse (ver. 13.7) TPS and is imported into our GEANT4 simulation together with the computed tomography (CT) data. The Hounsfield unit (HU) is converted into different material definitions using the method in Schneider *et al* (2000). The dose calculated using GEANT4 deviates slightly from TPS calculation due to differences in HU to stopping power conversion methodology and dose calculation algorithm (less than 3 mm biological range shift for 90% isodose line). However, this issue is inconsequential for relative comparison between RBE models in this work. Similar to the analysis done for clinical SOBP in the previous section, the RBE-weighted doses for the two cytoplasm materials are compared with the water. The metric for comparison is the *biological dose shift* which is the spatial change in isodose line of the RBE-weighted dose. The isodose level selected is 90%.

3. Results and discussion

The effect of cellular materials on depth-dose and LET_D profile are examined using GEANT4 and the results are shown in figure 2. The calculation is carried out with a SOBP with a range of 20 cm and modulation width of 5 cm in water. 5 mm thickness of cellular materials are placed at 17 cm depth near the centre of SOBP. The resulting dose and LET_D in figure 2 are quantities obtained along the central axis of the beam at different depths. Elevation in dose deposition and LET_D values are observed at positions where the cellular materials are present, indicating the choice of water as surrogate material will underestimate the local dose and LET_D. This suggests that the RBE model based on calculated LET_D may be modified after considering actual cellular materials composition. We will use cytoplasms (Incerti *et al* 2009, Byrne *et al* 2013) as the substitute for cellular material as this is the main composition of cell. Furthermore, there are studies that shows the cell-culture medium should have similar

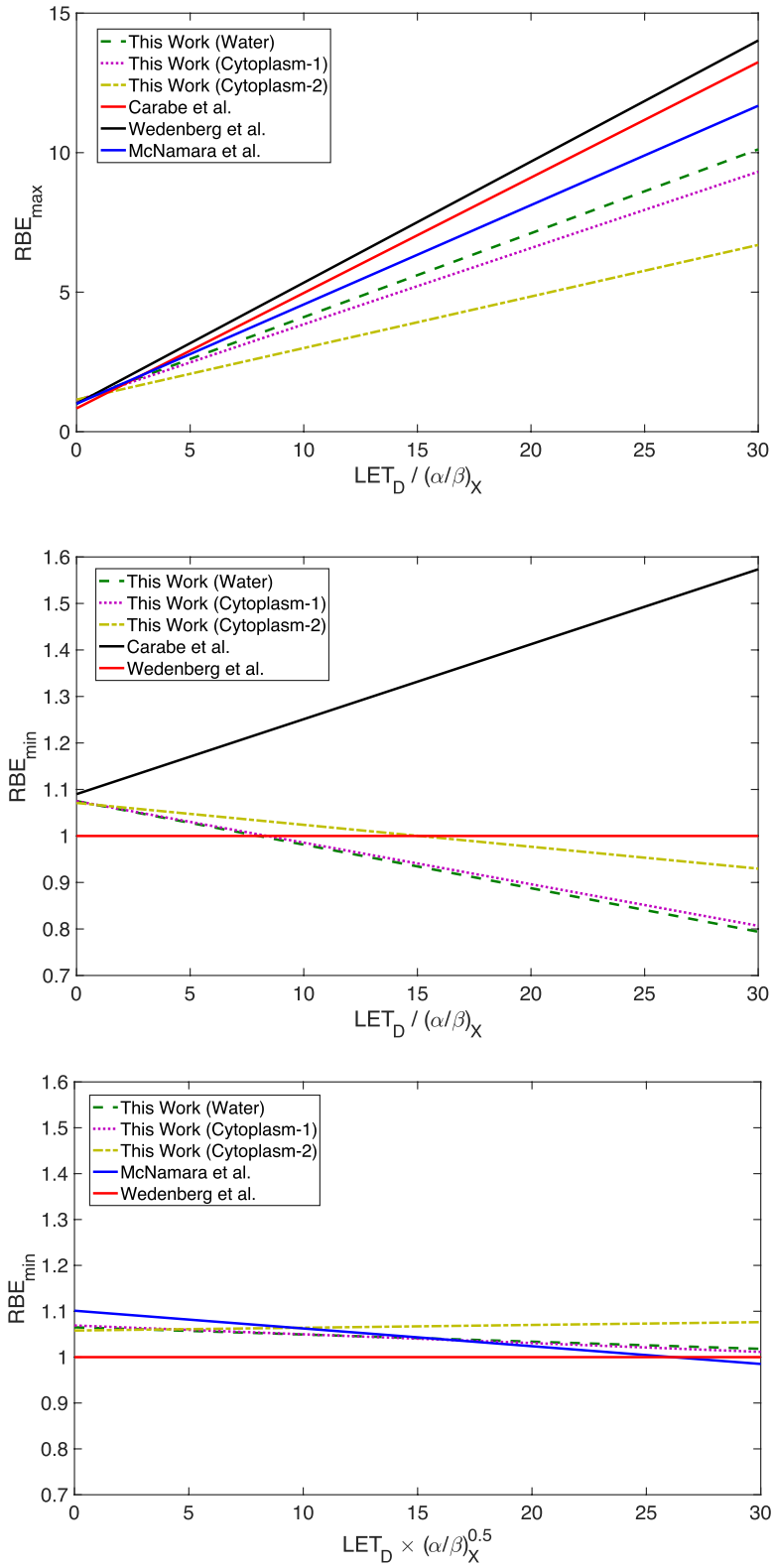

Figure 4. The solid lines represent the models obtained from Carabe *et al* (2012), Wedenberg *et al* (2013) and McNamara *et al* (2015) and the dashed, dotted and dashed-dotted lines are obtained from data assuming water, cytoplasm-1 and cytoplasm-2 materials respectively. (Top) A plot of RBE_{max} against $\mathrm{LET}_D/(\alpha/\beta)_X$. (Middle) A plot of RBE_{min} against $\mathrm{LET}_D/(\alpha/\beta)_X$. (Bottom) A plot of RBE_{max} against $\mathrm{LET}_D\sqrt{(\alpha/\beta)_X}$

elemental composition as the biological material (Spaargaren 1996). Hence, the cell-medium is assumed to be of the same materials of the cellular composition in this simulation.

The LET_D values were calculated for all *un-omitted* data points for three different materials—water, cytoplasm-1 (Incerti *et al* 2009) and cytoplasm-2 (Byrne *et al* 2013). These values are compiled and presented in the last three columns in appendix (The tables in Appendix A are sorted according to the type of cell lines. Table A1, A2, A3 and A4 corresponds to the Chinese Hamster, rat or mouse, human cancer and human

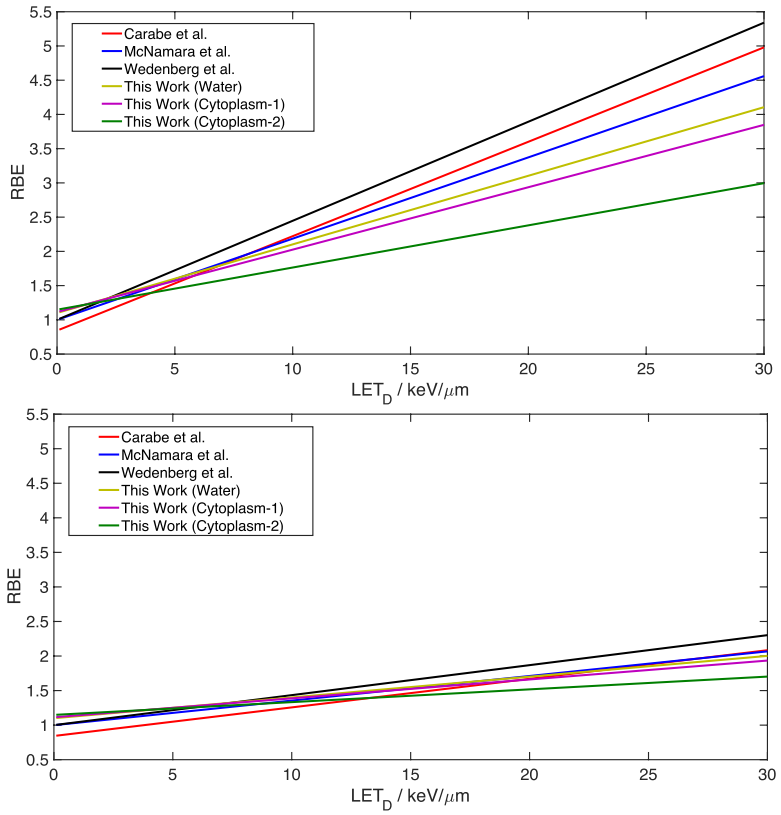

Figure 5. (Top) RBE plotted against LET_D for $(\alpha/\beta)_X = 3$. (Bottom) RBE plotted against LET_D for $(\alpha/\beta)_X = 10$.

$$RBE_{min} = p_0 + p_1 \frac{LET_D}{(\alpha/\beta)_X} \qquad RBE_{max} = p_2 + p_3 \frac{LET_D}{(\alpha/\beta)_X}$$

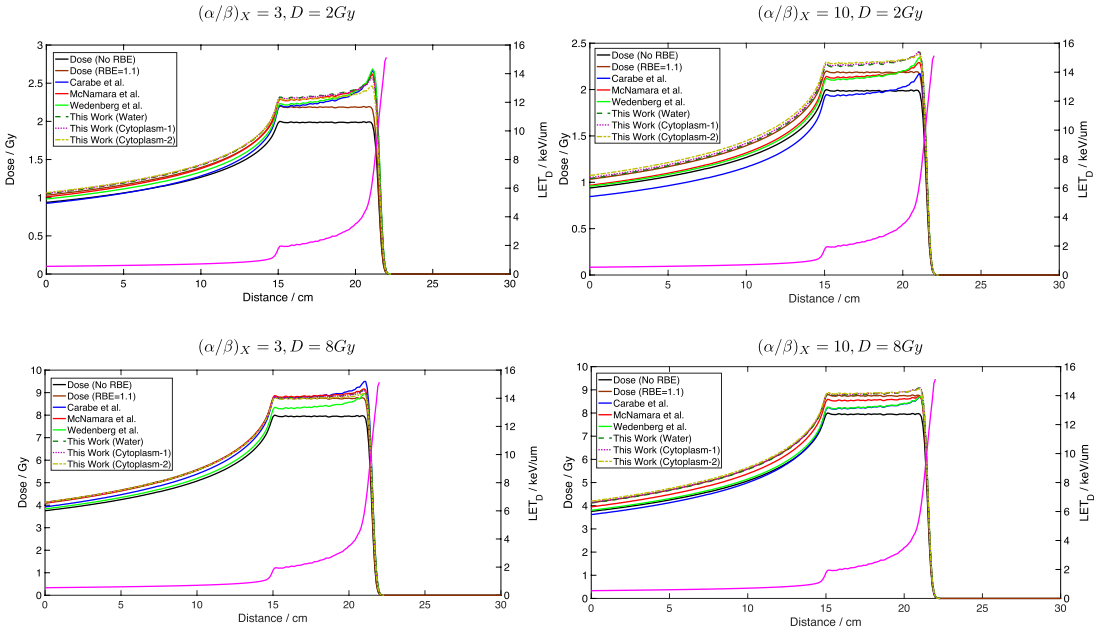

Figure 6. This figure shows the RBE weighted doses of a SOBP calculated using parameterization from equation (7) for (top left) $(\alpha/\beta)_X = 3, D = 2$ Gy, (top right) $(\alpha/\beta)_X = 19, D = 2$ Gy, (bottom left) $(\alpha/\beta)_X = 3, D = 8$ Gy, (bottom right) $(\alpha/\beta)_X = 10$, $D = 8$ Gy. The RBE-weighted dose are calculated for RBE models derived from 3 different cellular materials (shown in dotted, dashed, and dashed-dotted lines) and RBE models that are derived from Wedenberg *et al* (2013), Jones (2015) and McNamara *et al* (2015) (shown in solid lines). A constant RBE =1.1 calculation is also included in the graphs for comparison. The magenta line represents the LET_D with values shown on the right vertical axis.

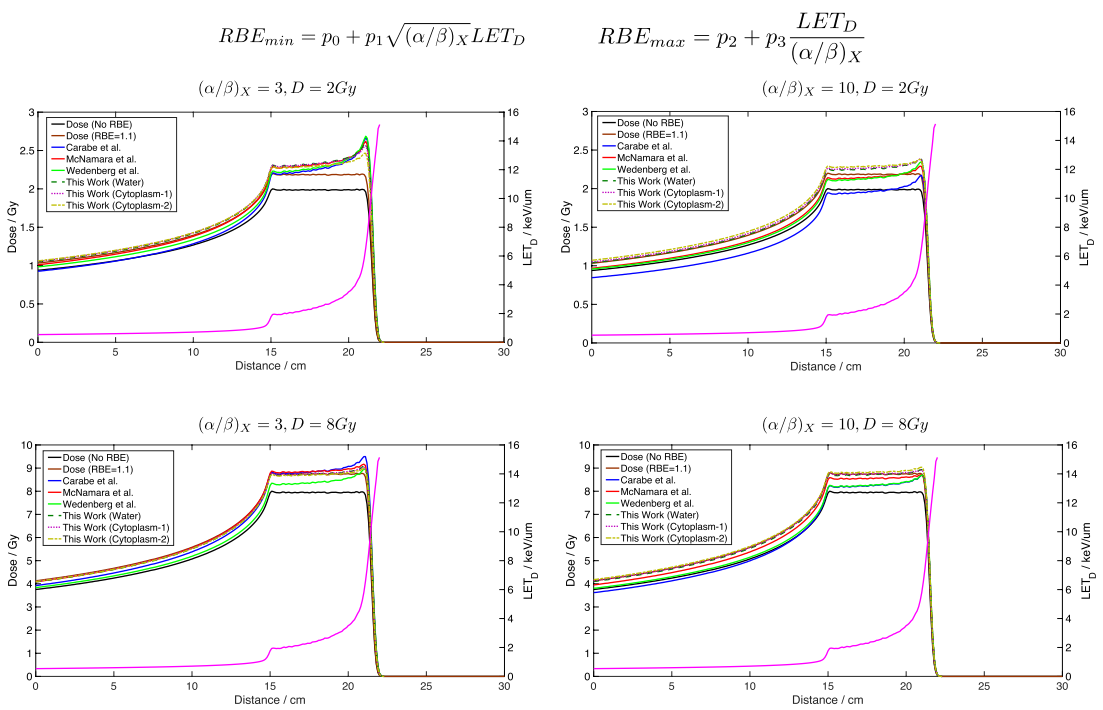

Figure 7. This figure shows the RBE weighted doses of a SOBP calculated using parameterization from equation (8) for (top left) $(\alpha/\beta)_X = 3, D = 2$ Gy, (top right) $(\alpha/\beta)_X = 19, D = 2$ Gy, (bottom left) $(\alpha/\beta)_X = 3, D = 8$ Gy, (bottom right) $(\alpha/\beta)_X = 10$, $D = 8$ Gy. The RBE-weighted dose are calculated for RBE models derived from 3 different cellular materials (shown in dotted, dashed, and dashed-dotted lines) and RBE models that are derived from Wedenberg *et al* (2013), Jones (2015) and McNamara *et al* (2015) (shown in solid lines). A constant RBE =1.1 calculation is also included in the graphs for comparison. The magenta line represents the LET$_D$ with values shown on the right vertical axis.

Table 2. A table of the summary of the results from figures 6 and 7. The percentage difference of D_{mean} and D_{max} are calculated from the SOBP between water and cytoplasm-1 and cytoplasm-2.

	RBE model from equation (7)				RBE model from equation (8)			
	Cytoplasm-1		Cytoplasm-2		Cytoplasm-1		Cytoplasm-2	
	ΔD_{mean} (%)	ΔD_{max} (%)	ΔD_{mean} (%)	ΔD_{max} (%)	ΔD_{mean} (%)	ΔD_{max} (%)	ΔD_{mean} (%)	ΔD_{max} (%)
$(\frac{\alpha}{\beta})_X = 3, D = 2$ Gy	0.32	0.94	1.83	4.35	0.23	0.95	1.71	4.02
$(\frac{\alpha}{\beta})_X = 10, D = 8$ Gy	−0.22	−0.00	−0.00	0.48	−0.27	0.11	−1.19	−1.15
$(\frac{\alpha}{\beta})_X = 3, D = 8$ Gy	0.11	0.36	0.72	1.63	−0.04	0.37	0.51	1.04
$(\frac{\alpha}{\beta})_X = 10, D = 2$ Gy	−0.40	0.01	−0.93	0.76	−0.42	0.06	−1.30	0.03

fibroblasts and epthelial cell lines, respectively). The classification of table A1, A2, A3 and A4 in appendix follows from Paganetti (2014) and should be referred upon for further information on the origin of the data. In general, the LET$_D$ values in appendix for cytoplasm-2 are larger than cytoplasm-1 which is in turn larger than water. Comparing different irradiation configurations as shown in figure 1, the greatest difference between our calculated LET$_D$ for water (column 4 in appendix A1) and the LET stated by Paganetti (2014) (column 7 in appendix A1) occurs for experiments involving low energy monoenergetic protons (configurations P1 and P2). The difference is about 17% with our calculated values being higher. It arises from an increased energy straggling effect from low energy proton (resulting in a distribution of primary protons energies) which is not accounted for when the journal reported the $pure-$ LET values. In contrast, the difference in LET$_D$ values for water at the SOBP region for configurations S1 and S2 is minimal at 5% as average LET in terms of LET$_D$ or LET$_t$ (instead of $pure-$ LET) are often reported in the journals or calculated by Paganetti *et al*.

3.1. Fitting results

The original data points from Paganetti (2014) and the corrected data points in our work with three different materials are shown in figure 3. This figure shows a plot of RBE$_{max}$ and RBE$_{min}$ against LET$_D/(\alpha/\beta)_X$ (due to the parameterisation used in equations (6)–(8)) and the regression lines are fitted using our data shown as blue circles. The regression lines are obtained using the least absolute residuals (LAR) algorithm (Dodge 2008) to reduce the effect of outliers which are present in figure 3. Greater rightward shifts can be observed for higher

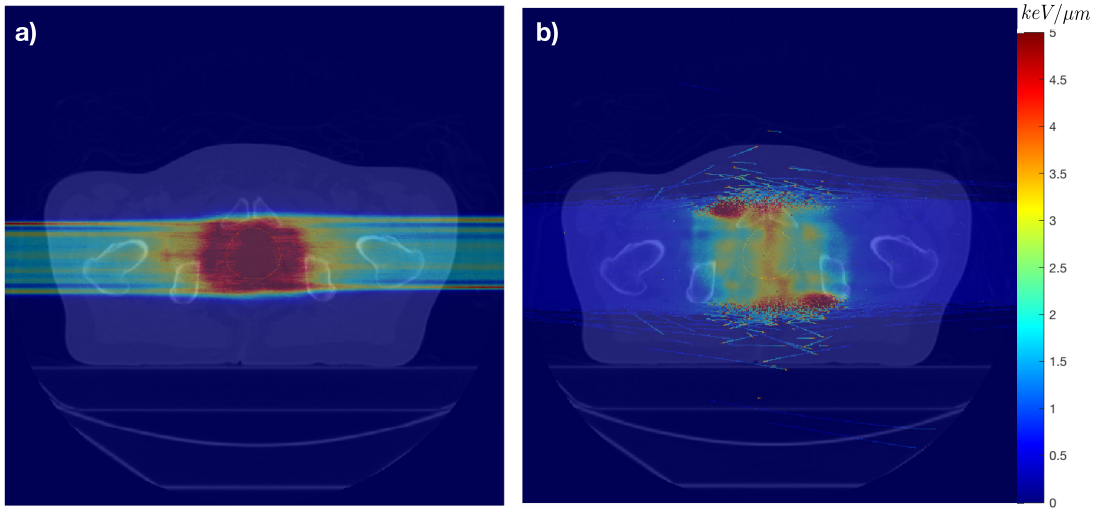

Figure 8. (a) This figure shows the dose calculation in a clinical prostate case with two opposing proton fields. The calculation is carried out using GEANT4 with HU to stopping power conversion following (Schneider *et al* 2000). (b) This figure shows the scoring of LET_D in the same clinical prostate case with two opposing fields.

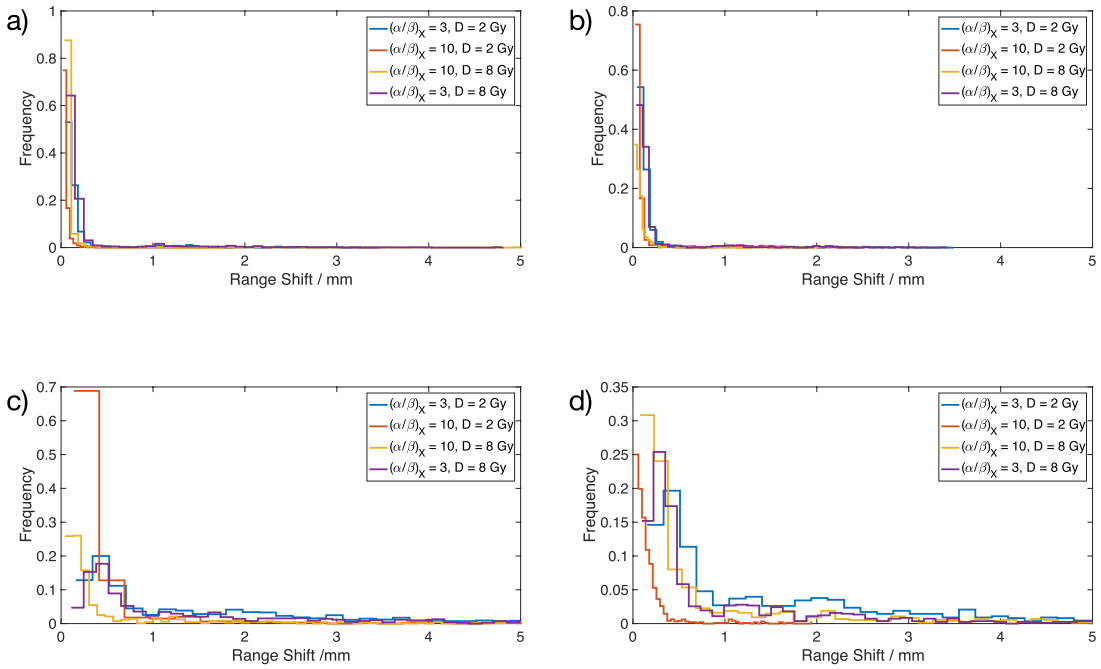

Figure 9. This figure shows the histograms of the range shifts of the 90% isodose contour between RBE-weighted dose of cytoplasmic materials and water under different model parameterisations. Graph (a) and (b) corresponds to RBE models derived from cytoplasm-1 and graph (c) and (d) corresponds to that from cytoplasm-2. Graph (a) and (c) use $RBE_{min} = p_0 + p_1 \frac{LET_D}{(\alpha/\beta)_X}$, $RBE_{max} = p_2 + p_3 \frac{LET_D}{(\alpha/\beta)_X}$, and graph (b) and (d) use $RBE_{min} = p_0 + p_1 LET_D \sqrt{(\alpha/\beta)_X}$, $RBE_{max} = p_2 + p_3 \frac{LET_D}{(\alpha/\beta)_X}$.

LET_D data as they usually arise from mono-energetic proton experiments which quote the *pure* − LET instead of LET_D values. Also, the rightward shift is greatest for cytoplasm-2 (from Byrne *et al* (2013)) data followed by cytoplasm-1 (from Incerti *et al* (2009)) data and lastly water data. The comparison of the regression lines for RBE_{min} and RBE_{max} for different materials are shown in figure 4. Three published models from Wedenberg *et al* (2013), Jones (2015) and McNamara *et al* (2015) are also plotted for comparison. The RBE_{min} data is plotted

against $\mathrm{LET}_D\sqrt{(\alpha/\beta)_X}$ in the bottom figure of figure 4 due to the parameterisation used by McNamara *et al* (2015) and Jones (2015). It can be seen that the RBE_{min} and RBE_{max} functions varied significantly between different *models* and between different assumed *cellular materials*. In general, a proper recalculation of LET_D in this work caused the regression lines to be *less steep* in figure 4, due to an increase in LET_D for most of the data points. The effect is most pronounced for cytoplasm-2 as the LET_D is considerably larger than water. Hence, this result shows that the inclusion of cellular materials in the simulation of irradiation experiment introduces a non-trivial and significant corrections to LET_D and the resulting RBE models.

Using the parameterisation given in equation (7), the RBE is calculated for different LET_D values for different cellular materials in figure 5. The top and bottom plots corresponds to $(\alpha/\beta)_X = 3$ and $(\alpha/\beta)_X = 10$ respectively. This figure shows that the RBE models calculated with LET_D re-calculation and with cellular materials addition deviate from the currently published one, and this applies to both normal tissue (low $(\alpha/\beta)_X$) and tumour (high $(\alpha/\beta)_X$). The deviation is maximal for cytoplasm-2 due to greatest difference of LET_D with water. Hence, the results in figure 5 suggests that the RBE values may in fact be *lower* than what is previously published and if true, can have an impact on treatment planning during proton therapy.

3.2. Results on SOBP

This section examines how the RBE models derived from different cellular materials affect a clinical-like SOBP. The results are shown in figures 6 and 7 which uses different RBE parameterisation according to equations (7) and (8) respectively. The LET_D of the SOBP are also shown in the same plot with the values stated in the right axis. The RBE-weighted SOBPs in figures 6 and 7 are calculated for $(\alpha/\beta)_X = 3$ and $(\alpha/\beta)_X = 10$, and for two different doses of 8 Gy and 2 Gy. Different RBE models from Wedenberg *et al* (2013), Jones (2015) and McNamara *et al* (2015) are also plotted in both figures to show how the *inter-model variation* compares with errors resulting from LET_D uncertainties. The summary of this comparison is shown in table 2. The difference in D_{mean} decreases with higher $(\alpha/\beta)_X$ and higher dose for all materials and all forms of parameterisations. The difference in D_{max} does not show a clear trend with dose but clearly decreases with higher $(\alpha/\beta)_X$ for both parameterisations. Also, the RBE-weighted doses are not equivalent between different RBE model parameterisations for all cellular materials. Thus, in practice, the choice of parameterisation will add another layer of uncertainty on top of the LET_D uncertainties from cellular materials. This result shows that the effect of LET_D uncertainties due to cytoplasmic cellular materials can lead up to 1.83% uncertainty in D_{mean} and 4.35% uncertainty in D_{max} for low $(\alpha/\beta)_X$ target under low dose (per fraction) such as in prostate cancer (van Leeuwen *et al* 2018). The uncertainties are negligible in all other cases with D_{mean} and D_{max} of about 1% or less.

3.3. Clinical case study

This section shows how the RBE models derived from different cellular materials affect a prostate clinical case. The tumor is contoured and shown as a white circle at the centre of figure 8. The dose and LET_D values are scored for each voxel for further calculation of RBE and RBE-weighted dose and is shown in figures 8(a) and (b) respectively. The result of this analysis is shown in the histograms in figure 9. The top two figures are calculated with cytoplasm-1 and the bottom two figures are calculated with cytoplasm-2 materials. The left two figures uses RBE model from equation (7) and the right two figures uses RBE model from equation (8). Across all the 4 histograms, the range shifts are higher for $(\alpha/\beta)_X = 3$ and $D = 2$ Gy data with a maximal *average* range shift of 1.6 mm, whereas the *average* range shifts are less than 1 mm for the rest of the data. This result is consistent with the SOBP study from the previous section and again, it shows that uncertainty in LET_D can have a significant dosimetric impact for low $(\alpha/\beta)_X$ and low dose values.

4. Conclusion

Our study shows that the cellular composition knowledge is critical under low $(\alpha/\beta)_X$ and low dose ($D \approx 2$ Gy) where mean and maximum dose uncertainties in SOBP can be up to 1.83% and 4.35% respectively, and the maximal range uncertainties in 90% isodose level can be up to 1.67 mm for our prostate clinical study. At the same time, the dose uncertainties are negligible for high $(\alpha/\beta)_X$ target and it is still valid to use water as a surrogate material for LET_D computation in this scenario. In overall, better dosimetry and precise instrumentation needs to be used (or developed) in future radiobiological experiments to obtain an accurate information of dose and LET_D values on biological targets to pave the way towards clinically robust RBE modelling.

Appendix. Calculated data used for this publication

Table A1. Calculated LET_D for Chinese hamster cell lines. The dashed line in the first column means $\beta_X = 0$.

$(\alpha/\beta)_x$ (Gy)	α (Gy^{-1})	β (Gy^{-2})	LET_D original (keV μm^{-1})	References	Configure	LET_D water (keV μm^{-1})	LET_D cytoplasm-1 (keV μm^{-1})	LET_D cytoplasm-2 (keV μm^{-1})
0	0.028	0.041	2.05	Hall *et al* (1978)	S2	1.94	1.99	2.31
0.5	0.024	0.033	4.3	Britten *et al* (1978)	S2	4.97	5.16	5.89
0.5	0.018	0.048	6.35	Britten *et al* (1978)	S2	7.34	7.71	9.93
0.5	0.016	0.033	8.22	Britten *et al* (1978)	S2	12.2	12.89	15.99
0.969	0.042	0.03	2.05	Hall *et al* (1978)	S2	1.94	1.99	2.31
0.969	0.038	0.029	1.11	Hall *et al* (1978)	S2	1.14	1.15	1.23
1.431	0.102	0.052	1.11	Wouters *et al* (2015)	S1	1.14	1.15	1.23
1.431	0.105	0.05	1.19	Wouters *et al* (2015)	S1	1.19	1.20	1.29
1.431	0.087	0.056	1.45	Wouters *et al* (2015)	S1	1.26	1.28	1.38
1.431	0.072	0.059	1.77	Wouters *et al* (2015)	S1	1.50	1.54	1.72
1.431	0.12	0.052	1.94	Wouters *et al* (2015)	S1	1.87	1.92	2.34
1.431	0.108	0.054	2.09	Wouters *et al* (2015)	S1	2.02	2.20	2.45
1.431	0.104	0.055	2.28	Wouters *et al* (2015)	S1	2.32	2.37	2.84
1.431	0.122	0.054	2.53	Wouters *et al* (2015)	S1	2.49	2.70	3.17
1.431	0.113	0.056	2.9	Wouters *et al* (2015)	S1	2.85	2.95	3.60
1.431	0.113	0.059	3.46	Wouters *et al* (2015)	S1	3.26	3.53	4.63
1.431	0.141	0.059	4.82	Wouters *et al* (2015)	S1	4.59	4.73	5.28
1.431	0.533	0.05	11	Wouters *et al* (2015)	S1	11.42	12.0	21.28
1.431	0.098	0.052	1.08	Wouters *et al* (2015)	S1	1.04	1.05	1.12
1.431	0.103	0.049	1.05	Wouters *et al* (2015)	S1	1.08	1.09	1.16
1.431	0.091	0.052	1.11	Wouters *et al* (2015)	S1	1.12	1.13	1.2
1.431	0.086	0.05	1.13	Wouters *et al* (2015)	S1	1.17	1.18	1.27
1.431	0.099	0.052	1.41	Wouters *et al* (2015)	S1	1.25	1.27	1.38
1.431	0.089	0.054	1.8	Wouters *et al* (2015)	S1	1.53	1.75	1.99
1.431	0.094	0.055	1.91	Wouters *et al* (2015)	S1	1.73	1.80	2.12
1.431	0.11	0.054	2.03	Wouters *et al* (2015)	S1	1.88	1.99	2.31
1.431	0.103	0.055	2.17	Wouters *et al* (2015)	S1	2.09	2.23	2.64
1.431	0.106	0.057	2.36	Wouters *et al* (2015)	S1	2.31	2.45	2.98
1.431	0.086	0.062	2.64	Wouters *et al* (2015)	S1	2.65	2.86	3.5
1.431	0.141	0.054	2.99	Wouters *et al* (2015)	S1	3.19	3.58	4.65
1.431	0.099	0.064	3.48	Wouters *et al* (2015)	S1	3.90	4.83	8
1.431	0.121	0.063	4.04	Wouters *et al* (2015)	S1	5.20	7.62	13.33
1.431	0.294	0.273	10.5	Wouters *et al* (2015)	S1	14.20	0.03	0
1.725	0.152	0.034	2.53	Grosse *et al* (2014)	S2	2.69	2.77	3.17
1.833	0.103	0.095	1.29	Gueulette *et al* (1996)	S2	1.28	1.3	1.49
1.833	0.192	0.077	4.1	Gueulette *et al* (1996)	S2	4	4.15	4.91
1.833	0.071	0.085	2.73	Gueulette *et al* (1996)	S2	2.98	3.03	3.35
1.833	0.198	0.073	4.53	Gueulette *et al* (1996)	S2	4.69	4.85	5.95
2.04	0.128	0.033	1.09	Coutrakon *et al* (1997)	S2	0.98	0.99	1.05
2.04	0.115	0.035	0.99	Coutrakon *et al* (1997)	S2	1	1.01	1.07
2.04	0.12	0.035	1.13	Coutrakon *et al* (1997)	S2	1.02	1.03	1.1
2.04	0.136	0.033	1.02	Coutrakon *et al* (1997)	S2	1.05	1.06	1.13
2.04	0.138	0.031	1.14	Coutrakon *et al* (1997)	S2	1.08	1.09	1.17
2.04	0.137	0.029	1.03	Coutrakon *et al* (1997)	S2	1.13	1.14	1.23
2.04	0.125	0.031	1.12	Coutrakon *et al* (1997)	S2	1.19	1.24	1.3
2.04	0.129	0.03	1.16	Coutrakon *et al* (1997)	S2	1.31	1.33	1.49
2.04	0.154	0.027	1.75	Coutrakon *et al* (1997)	S2	1.56	1.58	1.85
2.04	0.135	0.03	2.1	Coutrakon *et al* (1997)	S2	1.97	2.08	2.55
2.04	0.113	0.032	2.71	Coutrakon *et al* (1997)	S2	2.92	3.04	4.21
2.04	0.145	0.03	7.06	Coutrakon *et al* (1997)	S2	11.8	12.9	14.2
2.04	0.103	0.033	0.99	Coutrakon *et al* (1997)	S2	1.08	1.09	1.17

(*Continued*)

Table A1. (*Continued*)

$(\alpha/\beta)_x$ (Gy)	α (Gy^{-1})	β (Gy^{-2})	LET_D original (keV μm^{-1})	References	Configure	LET_D water (keV μm^{-1})	LET_D cytoplasm-1 (keV μm^{-1})	LET_D cytoplasm-2 (keV μm^{-1})
2.04	0.126	0.029	1.23	Coutrakon *et al* (1997)	S2	1.13	1.14	1.22
2.04	0.108	0.032	1.11	Coutrakon *et al* (1997)	S2	1.19	1.2	1.29
2.04	0.113	0.03	1.28	Coutrakon *et al* (1997)	S2	1.3	1.31	1.44
2.04	0.119	0.031	1.7	Coutrakon *et al* (1997)	S2	1.69	2.23	2.6
2.04	0.133	0.03	2.08	Coutrakon *et al* (1997)	S2	2.01	2.6	2.99
2.04	0.12	0.032	2.63	Coutrakon *et al* (1997)	S2	2.48	3.08	3.69
2.04	0.167	0.027	5.82	Coutrakon *et al* (1997)	S2	5.12	5.38	7.88
2.04	0.128	0.027	1.16	Coutrakon *et al* (1997)	S2	1.15	1.15	1.24
2.04	0.118	0.027	1.2	Coutrakon *et al* (1997)	S2	1.21	1.22	1.32
2.04	0.129	0.026	1.79	Coutrakon *et al* (1997)	S2	1.35	1.37	1.5
2.04	0.132	0.025	2.32	Coutrakon *et al* (1997)	S2	2.47	2.55	2.69
2.04	0.169	0.025	3.2	Coutrakon *et al* (1997)	S2	2.68	2.71	2.8
2.232	0.161	0.027	2.3	Wouters *et al* (1997)	S2	2.41	2.51	2.89
2.232	0.162	0.029	2.8	Wouters *et al* (1997)	S2	3.10	3.16	3.66
2.232	0.154	0.03	2.95	Wouters *et al* (1997)	S2	3.21	3.27	3.69
2.232	0.163	0.028	3.12	Wouters *et al* (1997)	S2	3.36	3.40	3.87
2.232	0.156	0.03	3.27	Wouters *et al* (1997)	S2	3.50	3.57	4.04
2.232	0.142	0.033	3.48	Wouters *et al* (1997)	S2	3.70	3.77	4.27
2.232	0.152	0.031	3.7	Wouters *et al* (1997)	S2	3.90	3.98	4.52
2.232	0.154	0.032	4	Wouters *et al* (1997)	S2	4.19	4.27	4.86
2.232	0.139	0.035	4.4	Wouters *et al* (1997)	S2	4.57	4.66	5.31
2.232	0.143	0.035	5.28	Wouters *et al* (1997)	S2	5.47	5.60	6.39
2.232	0.17	0.034	6.3	Wouters *et al* (1997)	S2	6.41	6.57	7.56
2.626	0.213	0.04	1.18	Raju *et al* (1978b)	S1	1.231	1.242	1.354
2.626	0.091	0.049	2.12	Raju *et al* (1978b)	S1	2.16	2.23	2.95
2.783	0.744	0	30.5	Belli *et al* (1993)	P2	38.25	39.96	53.10
2.783	0.471	0.044	20	Belli *et al* (1993)	P2	21.73	22.54	26.81
2.783	0.372	0.036	10.9	Belli *et al* (1993)	P2	11.31	11.72	13.58
2.804	0.653	0	34.6	Belli *et al* (1998)	P2	37.70	39.33	51.82
2.804	0.721	0	30.5	Belli *et al* (1998)	P2	32.88	34.04	42.68
2.804	0.469	0.043	20	Belli *et al* (1998)	P2	20.78	21.57	25.57
2.804	0.372	0.036	11	Belli *et al* (1998)	P2	11.20	11.61	13.45
2.804	0.289	0.024	7.7	Belli *et al* (1998)	P2	7.96	8.19	9.43
2.804	0.3	0.052	22.91	Goodhead *et al* (1992)	P2	24.33	25.23	30.29
2.804	0.42	0.019	20.27	Goodhead *et al* (1992)	P2	21.36	22.17	26.33
3.018	0.115	0.049	2.53	Grosse *et al* (2014)	S2	2.69	2.77	3.17
3.305	0.1	0.025	5.22	Yashkin *et al* (1995)	S1	5.25	5.29	5.67
3.305	0.071	0.03	1.09	Yashkin *et al* (1995)	S2	1.015	1.020	1.089
3.429	0.204	0.021	11	Matsumura *et al* (1999)	P2	11.23	11.64	13.48
3.429	0.226	0.02	4.25	Matsumura *et al* (1999)	P2	4.42	4.52	5.14
3.429	0.151	0.025	1.25	Matsumura *et al* (1999)	P2	1.27	1.30	1.48
3.652	0.329	0.024	3.71	Blomquist *et al* (1993)	S2	3.91	3.99	4.53
3.905	0.109	0.016	0.79	Wainson *et al* (1972)	P2	0.810	0.820	0.926
3.905	0.243	0.013	3.1	Wainson *et al* (1972)	P2	14.17	14.74	17.4
4.045	0.085	0.04	2.53	Grosse *et al* (2014)	S2	2.69	2.77	3.17
4.056	0.399	0.272	9.23	Bird *et al* (1980)	P2	9.51	9.83	11.1
4.074	1.03	0	32	Folkard *et al* (1989)	P2	38.25	40.0	53.10
4.074	0.33	0.066	24	Folkard *et al* (1989)	P2	25.79	26.69	32.22
4.074	0.11	0.027	17	Folkard *et al* (1989)	P2	17.12	17.82	20.90
4.074	0.35	0.045	17	Prise *et al* (1990)	P1	31.8	33.8	38.99
4.074	0.33	0.066	24	Prise *et al* (1990)	P1	39.7	42.63	49.58
4.074	1.03	0	32	Prise *et al* (1990)	P1	46.6	50.09	61.8

(*Continued*)

Table A1. (*Continued*)

$(\alpha/\beta)_x$ (Gy)	α (Gy^{-1})	β (Gy^{-2})	LET_D original (keV μm^{-1})	References	Configure	LET_D water (keV μm^{-1})	LET_D cytoplasm-1 (keV μm^{-1})	LET_D cytoplasm-2 (keV μm^{-1})
4.338	0.062	0.023	1.22	Robertson *et al* (1994)	P2	1.24	1.26	1.43
4.338	0.071	0.025	1.12	Robertson *et al* (1994)	P2	1.13	1.15	1.31
4.338	0.053	0.037	1.91	Robertson *et al* (1994)	P2	1.94	1.98	2.25
4.338	0.062	0.04	2.41	Robertson *et al* (1994)	P2	2.47	2.52	2.87
4.338	0.044	0.047	3.83	Robertson *et al* (1994)	P2	4.07	4.15	4.90
4.338	0.09	0.019	1.17	Robertson *et al* (1994)	P2	1.19	1.21	1.37
4.338	0.096	0.019	1.12	Robertson *et al* (1994)	P2	1.13	1.15	1.31
4.338	0.068	0.023	1.07	Robertson *et al* (1994)	P2	1.09	1.10	1.25
4.338	0.083	0.024	1.19	Robertson *et al* (1994)	P2	1.21	1.23	1.39
4.338	0.079	0.033	1.69	Robertson *et al* (1994)	P2	1.72	1.74	1.99
4.338	0.081	0.04	2.06	Robertson *et al* (1994)	P2	2.10	2.14	2.44
4.338	0.05	0.046	2.54	Robertson *et al* (1994)	P2	2.61	2.66	3.03
4.338	0.06	0.048	6.08	Robertson *et al* (1994)	P2	6.19	6.35	7.30
4.338	0.099	0.019	1.07	Robertson *et al* (1994)	P2	1.09	1.10	1.25
4.338	0.099	0.02	1.08	Robertson *et al* (1994)	P2	1.10	1.11	1.26
4.338	0.073	0.022	0.99	Robertson *et al* (1994)	P2	1.01	1.02	1.16
4.338	0.087	0.02	1.08	Robertson *et al* (1994)	P2	1.10	1.11	1.26
4.338	0.07	0.021	1.05	Robertson *et al* (1994)	P2	1.07	1.08	1.22
4.338	0.047	0.024	1.09	Robertson *et al* (1994)	P2	1.10	1.11	1.26
4.338	0.04	0.024	1.16	Robertson *et al* (1994)	P2	1.19	1.21	1.37
4.338	0.037	0.025	1.13	Robertson *et al* (1994)	P2	1.13	1.15	1.31
4.338	0.042	0.028	1.72	Robertson *et al* (1994)	P2	1.75	1.78	2.02
4.338	0.027	0.039	1.88	Robertson *et al* (1994)	P2	1.91	1.95	2.22
4.338	0.019	0.043	2.76	Robertson *et al* (1994)	P2	2.84	2.89	3.30
4.338	0.04	0.037	7.75	Robertson *et al* (1994)	P2	7.88	8.11	9.33
4.522	0.142	0.032	2.53	Grosse *et al* (2014)	S2	2.69	2.77	3.17
5	0.14	0.045	3.44	Schuff *et al* (2002)	S2	3.84	4.03	4.62
5.054	0.122	0.149	9.23	Bird *et al* (1980)	P2	9.51	9.83	11.1
5.217	0.12	0.067	17.6	Jeynes *et al* (2012)	P1	31.48	33.67	38.63
5.833	0.221	0.041	6.23	Moertel *et al* (2004)	P1	15.51	16.39	19.21
6.52	0.199	0.022	1.74	Tang *et al* (1997)	S2	1.81	1.84	2.10
6.52	0.207	0.025	3.11	Tang *et al* (1997)	S2	3.36	3.42	3.86
6.52	0.217	0.026	3.79	Tang *et al* (1997)	S2	4.09	4.17	4.74
6.52	0.249	0.025	4.76	Tang *et al* (1997)	S2	4.91	5.02	5.73
7.667	0.539	0	27.6	Sgura *et al* (2000)	P1	42.22	46.13	56.52
7.667	0.194	0.023	7.7	Sgura *et al* (2000)	P1	18.97	19.99	23.51
8.571	0.12	0.028	1.18	Raju *et al* (1978a)	S1	1.231	1.242	1.354
8.571	0.065	0.033	2.12	Raju *et al* (1978a)	S1	2.16	2.23	2.95
10.814	0.72	0.03	2.53	Grosse *et al* (2014)	S2	2.69	2.77	3.17
11.724	0.116	0.007	17	Prise *et al* (1990)	P1	31.8	33.8	38.99
11.724	0.239	0.005	24	Prise *et al* (1990)	P1	39.7	42.63	49.58
11.724	0.561	0	32	Prise *et al* (1990)	P1	46.6	50.09	61.8
13.061	0.214	0.021	2.02	Ando *et al* (2001)	S2	2.02	2.12	4.38
13.061	0.166	0.024	1.02	Ando *et al* (2001)	S2	1.05	1.06	1.14
13.971	0.172	0.039	2.53	Grosse *et al* (2014)	S2	2.69	2.77	3.17
15.152	0.07	0.001	1.18	Raju *et al* (1978a)	S1	1.231	1.242	1.354
15.152	0.034	0.003	2.12	Raju *et al* (1978a)	S1	2.16	2.23	2.95
16.296	0.345	0.035	2.53	Grosse *et al* (2014)	S2	2.69	2.77	3.17
18.432	0.875	0.023	2.53	Grosse *et al* (2014)	S2	2.69	2.77	3.17
25.455	0.43	0	12.1	Perris *et al* (1986)	P2	11.90	12.34	14.31
25.455	0.21	0.023	5.8	Perris *et al* (1986)	P2	5.91	6.05	6.93
91.509	1.708	0.005	2.53	Grosse *et al* (2014)	S2	2.69	2.77	3.17
114.9	0.99	0.017	2.53	Grosse *et al* (2014)	S2	2.69	2.77	3.17

Table A2. Calculated LET_D for rat or mouse cell lines.

$(\alpha/\beta)_x$ (Gy)	α (Gy^{-1})	β (Gy^{-2})	LET_D original (keV μm^{-1})	References	Configure	LET_D water (keV μm^{-1})	LET_D cytoplasm-1 (keV μm^{-1})	LET_D cytoplasm-2 (keV μm^{-1})
0	0.093	0.024	0.42	Green *et al* (2001)	P2	0.45	0.46	0.51
0	0.088	0.013	0.42	Green *et al* (2001)	P2	0.45	0.46	0.51
0	0	0.011	1.21	Williams *et al* (1978)	S2	1.23	1.25	1.42
0	0.028	0.036	1.21	Williams *et al* (1978)	S2	1.23	1.25	1.42
0	0.036	0.004	1.21	Williams *et al* (1978)	S2	1.23	1.25	1.42
0.051	0.14	0.044	2.69	Schuff *et al* (2002)	P2	2.88	2.92	3.29
0.051	0.32	0.06	14	Schuff *et al* (2002)	P2	12.22	12.68	14.71
0.051	0.67	0.23	26	Schuff *et al* (2002)	P2	21.86	22.67	26.97
1.871	0.121	0.023	0.42	Green *et al* (2001)	P2	0.45	0.46	0.51
2.174	0.073	0.049	3.4	Ibanez *et al* (2009)	P2	3.61	3.68	4.17
2.174	0.61	0	14	Ibanez *et al* (2009)	P2	12.22	12.68	14.71
3.514	0.31	0.02	3.1	Schuff *et al* (2002)	P2	3.19	3.25	3.67
3.514	0.32	0.05	15.6	Schuff *et al* (2002)	P2	13.74	14.28	16.62
3.514	0.69	0.26	26	Schuff *et al* (2002)	P2	21.86	22.67	26.97
4.39	0.31	0.03	10	Hei *et al* (1988)	P2	10.2	10.56	12.21
5.6	0.084	0.002	0.42	Green *et al* (2002)	P2	0.45	0.46	0.51
5.965	0.43	0.013	22.91	Goodhead *et al* (1992)	P2	24.33	25.23	30.29
5.965	0.43	0.013	20.27	Goodhead *et al* (1992)	P2	21.36	22.17	26.33
7.009	0.017	0.053	1.21	Robertson *et al* (1975)	S2	0.816	0.827	0.934
7.009	0	0.04	1.45	Robertson *et al* (1975)	S2	0.976	0.990	1.13
7.009	0.071	0.039	2.28	Robertson *et al* (1975)	S2	2.24	2.30	2.64
7.009	0.139	0.044	3.05	Robertson *et al* (1975)	S2	3.09	3.20	3.63
7.009	0	0.054	3.51	Robertson *et al* (1975)	S2	4.62	4.64	5.98
7.625	0.097	0.022	0.42	Green *et al* (2002)	P2	0.45	0.46	0.51
7.854	0.226	0.044	1.21	Williams *et al* (1978)	S2	1.23	1.25	1.42
8.032	0.212	0.025	2.2	Urano *et al* (1980)	S2	2.13	2.16	2.46
11.646	0.211	0.017	2.2	Urano *et al* (1980)	S2	2.13	2.16	2.46
11.646	0.203	0.021	1.19	Urano *et al* (1980)	S2	1.16	1.17	1.25
14	0.079	0.006	0.42	Green *et al* (2002)	P2	0.45	0.46	0.51
15	0.47	0.019	11	Bettega *et al* (1998)	P2	11.37	11.78	13.65
15	0.43	0.038	19.7	Bettega *et al* (1998)	P2	21.12	21.92	26.02
15	0.55	0.053	28.8	Bettega *et al* (1998)	P2	33.25	34.43	43.30
15	0.67	0	31.6	Bettega *et al* (1998)	P2	37.70	39.33	51.82
15	0.75	0	32.5	Bettega *et al* (1998)	P2	39.40	41.32	56.18
15	1.02	0	33.2	Bettega *et al* (1998)	P2	40.66	42.87	59.72
15	0.05	0.041	1.83	Bettega *et al* (1990)	P2	2.27	2.30	2.55
19.706	0.282	0.021	2.2	Urano *et al* (1980)	S2	2.13	2.16	2.46
29.448	0.195	0.005	3.94	Satoh *et al* (1986)	S2	4.13	4.21	4.79
39.583	0.114	0.004	2.2	Urano *et al* (1980)	S2	2.13	2.16	2.46
49.5	0.075	0.004	0.42	Green *et al* (2002)	P2	0.45	0.46	0.51
218.61	0.287	0.004	11	Sakamoto *et al* (1980)	P2	10.78	8.47	9.11
—	0.402	0	11	Sakamoto *et al* (1980)	P2	10.78	8.47	9.11
—	0.411	0	1.25	Sakamoto *et al* (1980)	P2	1.30	1.33	1.52

Table A3. Calculated LET_D for human cancer cell lines.

$(\alpha/\beta)_x$ (Gy)	α (Gy^{-1})	β (Gy^{-2})	LET_D original (keV μm^{-1})	References	Configure	LET_D water (keV μm^{-1})	LET_D cytoplasm-1 (keV μm^{-1})	LET_D cytoplasm-2 (keV μm^{-1})
0.577	0.034	0.098	2.56	Aoki-Nakano (2014)	S2	2.38	2.46	2.85
0.695	0.82	0.73	2.2	Gerelchuluun *et al* (2011)	S2	2.53	2.62	3.08
0.754	0.215	0.058	2.6	Aoki-Nakano (2014)	S2	2.38	2.46	2.85
0.992	0	0.114	2.56	Aoki-Nakano (2014)	S2	2.38	2.46	2.85
1.46	0.473	0.085	2.02	Ando *et al* (2001)	S2	2.02	2.12	4.38
1.46	0.245	0.097	1.02	Ando *et al* (2001)	S2	1.05	1.06	1.14
1.833	0.14	0.064	1.11	Chaudhary *et al* (2014)	P2	1.11	1.13	1.29
1.833	0.17	0.065	4.02	Chaudhary *et al* (2014)	P2	4.02	4.50	5.16
1.833	0.22	0.071	7	Chaudhary *et al* (2014)	P2	7	7.33	8.42
1.833	0.44	0.045	11.9	Chaudhary *et al* (2014)	P2	11.9	12.61	14.63
1.833	0.77	0.008	18	Chaudhary *et al* (2014)	P2	18	19.62	23.12
1.833	0.9	0.01	22.6	Chaudhary *et al* (2014)	P2	22.6	25.23	30.29
1.833	0.16	0.056	1.2	Chaudhary *et al* (2014)	S2	1.2	1.250	1.42
1.833	0.19	0.058	2.6	Chaudhary *et al* (2014)	S2	2.6	2.64	3.04
1.833	0.22	0.064	4.5	Chaudhary *et al* (2014)	S2	4.5	4.76	5.43
1.833	0.31	0.056	13.4	Chaudhary *et al* (2014)	S2	13.4	14.24	16.56
1.833	0.41	0.056	21.7	Chaudhary *et al* (2014)	S2	21.7	22.7	26.97
1.833	0.5	0.064	25.9	Chaudhary *et al* (2014)	S2	25.9	26.7	32.2
1.851	0.036	0.01	2.42	Calugaru *et al* (2011)	S2	2.62	2.67	3.07
1.851	0.029	0.012	4.04	Calugaru *et al* (2011)	S2	4.22	4.31	4.90
1.851	0.027	0.016	6.85	Calugaru *et al* (2011)	S2	6.98	7.17	8.23
1.851	0.044	0.009	1.14	Calugaru *et al* (2011)	S2	1.120	1.121	1.140
1.851	0.044	0.009	2.74	Calugaru *et al* (2011)	S2	2.63	2.72	3.12
1.851	0.044	0.009	5.8	Calugaru *et al* (2011)	S2	5.43	5.63	6.53
2.078	0.85	0.037	22.91	Goodhead *et al* (1992)	P2	24.33	25.23	30.29
2.078	0.53	0.084	20.27	Goodhead *et al* (1992)	P2	21.36	22.17	26.33
2.392	0.054	0.017	3.94	Satoh *et al* (1986)	S2	4.13	4.21	4.79
2.511	0.104	0.042	1.3	Courdi *et al* (1994)	S2	1.33	1.35	1.54
2.511	0.057	0.057	2.3	Courdi *et al* (1994)	S2	2.46	2.51	2.89
2.511	0.048	0.062	2.65	Courdi *et al* (1994)	S2	2.94	2.99	3.37
2.511	0.035	0.072	3.75	Courdi *et al* (1994)	S2	3.95	4.03	4.57
2.511	0.096	0.074	6	Courdi *et al* (1994)	S2	6.12	6.27	7.18
2.998	0.385	0.08	2.5	Zhang *et al* (2013)	S2	2.57	2.62	2.98
3.083	0.47	0	7.7	Baggio *et al* (2002)	P2	7.84	8.07	9.29
3.763	0.344	0.044	5.1	Britten *et al* (1978)	S2	4.99	5.26	6.23
3.763	0.385	0.051	7.6	Britten *et al* (1978)	S2	8.01	8.40	10.28
3.763	0.372	0.063	8.72	Britten *et al* (1978)	S2	10.1	10.63	12.63
3.763	0.166	0.058	2.66	Britten *et al* (1978)	S2	2.95	3.00	3.38
3.763	0.286	0.044	4.3	Britten *et al* (1978)	S2	4.47	4.56	5.19
3.763	0.598	0.017	6.35	Britten *et al* (1978)	S2	6.46	6.63	7.63
3.763	0.555	0.053	8.22	Britten *et al* (1978)	S2	8.34	8.60	9.90
3.891	0.158	0.077	13.5	Inada *et al* (1981)	P2	13.93	10.58	16.63
3.891	0.048	0.088	1.3	Inada *et al* (1981)	P2	1.25	1.27	1.45
4	0.15	0.03	2.2	Gerelchuluun *et al* (2011)	S2	2.53	2.62	3.08
4.056	0.168	0.053	1.11	Kagawa *et al* (2001)	S2	1.028	1.033	1.103
4.056	0.18	0.051	2.11	Kagawa *et al* (2001)	S2	1.95	2.01	2.21
4.056	0.176	0.052	2.54	Kagawa *et al* (2001)	S2	2.38	2.46	2.86
4.056	0.155	0.06	3.91	Kagawa *et al* (2001)	S2	3.61	3.74	4.37
4.056	0.17	0.057	4.8	Kagawa *et al* (2001)	S2	4.21	4.25	4.98
4.455	0.157	0.048	1.2	Kase *et al* (2013)	S2	1.192	1.20	1.29
4.455	0.164	0.048	2.03	Kase *et al* (2013)	S2	1.64	1.69	2.06

(*Continued*)

Table A3. (*Continued*)

$(\alpha/\beta)_x$ (Gy)	α (Gy^{-1})	β (Gy^{-2})	LET_D original (keV μm^{-1})	References	Configure	LET_D water (keV μm^{-1})	LET_D cytoplasm-1 (keV μm^{-1})	LET_D cytoplasm-2 (keV μm^{-1})
4.455	0.139	0.056	2.65	Kase *et al* (2013)	S2	2.52	2.62	3.10
4.455	0.141	0.062	4.25	Kase *et al* (2013)	S2	4.29	4.44	5.24
4.455	0.237	0.049	0.56	Matsuura *et al* (2010)	P2	0.590	0.596	0.670
4.455	0.348	0.047	3.19	Matsuura *et al* (2010)	P2	3.674	3.76	4.38
4.455	0.183	0.053	0.56	Matsuura *et al* (2010)	P2	0.590	0.596	0.670
4.455	0.294	0.056	3.19	Matsuura *et al* (2010)	P2	3.674	3.76	4.38
4.604	0.315	0.038	2.5	Aoki-Nakano (2014)	S2	2.38	2.46	2.85
4.999	0.273	0.046	1.14	Baek *et al* (2008)	S2	1.072	1.072	1.145
4.999	0.291	0.041	2.56	Baek *et al* (2008)	S2	2.43	2.49	2.91
5.803	0.086	0.033	2.86	Butterworth (2013)	S2	2.85	2.96	3.47
5.803	0.147	0.021	2.86	Butterworth (2013)	S2	2.85	2.96	3.47
6.023	1.01	0	22.91	Goodhead *et al* (1992)	P2	24.33	25.23	30.29
6.023	0.67	0.037	20.27	Goodhead *et al* (1992)	P2	21.36	22.17	26.33
6.333	0.22	0.05	2.76	Matsumoto *et al* (2014)	S2	2.70	2.80	3.10
6.333	0.28	0.05	3.27	Matsumoto *et al* (2014)	S2	3.17	3.28	3.79
6.333	0.25	0.05	3.615	Matsumoto *et al* (2014)	S2	3.61	3.74	4.37
6.333	0.26	0.05	4.07	Matsumoto *et al* (2014)	S2	3.99	4.13	4.84
6.333	0.42	0.05	4.93	Matsumoto *et al* (2014)	S2	4.59	4.75	5.59
6.333	0.41	0.05	6.19	Matsumoto *et al* (2014)	S2	5.83	6.06	7.20
6.333	0.38	0.05	7.9	Matsumoto *et al* (2014)	S2	8.93	9.44	12.44
6.333	0.44	0.03	9.445	Matsumoto *et al* (2014)	S2	13.17	13.85	16.54
6.333	0.42	0.03	10.8	Matsumoto *et al* (2014)	S2	16.46	17.31	20.71
6.343	0.504	0.047	6.23	Moertel *et al* (2004)	P1	15.51	16.39	19.21
6.897	0.253	0.024	2.42	Calugaru *et al* (2011)	S2	2.62	2.67	3.07
6.897	0.293	0.02	4.04	Calugaru *et al* (2011)	S2	4.22	4.31	4.90
6.897	0.352	0.021	6.85	Calugaru *et al* (2011)	S2	6.98	7.17	8.23
6.897	0.213	0.054	1.14	Calugaru *et al* (2011)	S2	1.120	1.121	1.14
6.897	0.213	0.054	2.74	Calugaru *et al* (2011)	S2	2.63	2.72	3.12
6.897	0.213	0.054	5.8	Calugaru *et al* (2011)	S2	5.43	5.63	6.53
7.111	0.188	0.038	0.79	Wainson *et al* (1972)	P2	1.98	1.99	2.08
7.613	0.218	0.039	2.56	Ogata *et al* (2005)	S2	2.38	2.46	2.86
7.647	0.15	0.011	7.7	Belli *et al* (2000)	P2	8.01	8.24	9.49
7.647	0.23	0.004	19.8	Belli *et al* (2000)	P2	20.78	21.57	25.57
7.647	0.57	0	30	Belli *et al* (2000)	P2	32.88	34.04	42.68
10.893	0.243	0.041	17.1	Yogo *et al* (2011)	P1	29.12	30.93	38.29
11.333	0.11	0.024	1.93	Bettega *et al* (2000)	S2	2.02	2.06	2.36
11.333	0.19	0.026	2.42	Bettega *et al* (2000)	S2	2.62	2.67	3.07
11.333	0.14	0.039	6.71	Bettega *et al* (2000)	S2	6.84	7.03	8.07
11.333	0.15	0.032	8.11	Bettega *et al* (2000)	S2	8.23	8.48	9.77
11.333	0.34	0.15	8.83	Bettega *et al* (2000)	S2	8.97	9.26	10.7
16.493	0.389	0.027	2.5	Zhang *et al* (2013)	S2	2.72	2.77	3.19
17.1	0.03	0.002	6.2	Pertrovic *et al* (2006)	P2	5.32	5.44	6.220
17.1	0.03	0.002	6.9	Ristic-Fira *et al* (2008)	P2	5.28	5.400	6.17
18.387	0.41	0.092	7.7	Belli *et al* (2000)	P2	8.01	8.24	9.49
18.387	0.87	0	19.7	Belli *et al* (2000)	P2	20.78	21.57	25.57
18.387	0.81	0	29.5	Belli *et al* (2000)	P2	32.88	34.04	42.68
18.444	0.824	0	10	Wera *et al* (2013)	P2	9.70	10.020	11.58
18.444	1.26	0	25.5	Wera *et al* (2011)	P2	30.510	31.56	38.92
47.5	0.57	0.012	1.93	Bettega *et al* (2000)	S2	2.02	2.06	2.36
47.5	0.61	0.01	2.42	Bettega *et al* (2000)	S2	2.62	2.67	3.07
47.5	0.7	0.018	6.71	Bettega *et al* (2000)	S2	6.84	7.03	8.07
47.5	0.83	0.001	8.11	Bettega *et al* (2000)	S2	8.23	8.48	9.77

(*Continued*)

Table A3. (*Continued*)

$(\alpha/\beta)_x$ (Gy)	α (Gy^{-1})	β (Gy^{-2})	LET_D original (keV μm^{-1})	References	Configure	LET_D water (keV μm^{-1})	LET_D cytoplasm-1 (keV μm^{-1})	LET_D cytoplasm-2 (keV μm^{-1})
47.5	1.23	0	8.83	Bettega *et al* (2000)	S2	8.97	9.26	10.7
51.805	0.053	0.001	9.08	Ristic-Fira *et al* (2010)	P2	9.25	9.55	1.10
51.805	0.082	0	4.71	Ristic-Fira *et al* (2010)	S2	4.78	4.90	5.60
69.5	0.32	0.06	7.7	Baggio *et al* (2002)	P2	7.84	8.07	9.29
—	0.653	0.004	3.71	Blomquist *et al* (1993)	S2	3.91	3.99	4.53
—	0.061	0	2.6	Pertrovic *et al* (2010)	S2	1.92	1.93	2.10
—	0.073	0	4.7	Pertrovic *et al* (2010)	S2	2.14	2.17	2.39
—	0.104	0	10.1	Pertrovic *et al* (2010)	S2	4.19	4.28	4.86
—	0.267	0	18.8	Pertrovic *et al* (2010)	S2	6.96	7.15	8.21
—	0.101	0	4.7	Keta *et al* (2014)	S2	4.78	4.90	5.60

Table A4. Calculated LET_D for human fibroblasts and epithelial cell lines.

$(\alpha/\beta)_x$ (Gy)	α (Gy^{-1})	β (Gy^{-2})	LET_D original (keV μm^{-1})	References	Configure	LET_D water (keV μm^{-1})	LET_D cytoplasm-1 (keV μm^{-1})	LET_D cytoplasm-2 (keV μm^{-1})
2.31	0.694	0.011	10	Hei *et al* (2014)	P2	10.2	10.56	12.21
6.862	0.5	0.054	2.25	Slonina *et al* (2014)	S2	2.25	2.29	2.54
6.862	0.493	0.054	2.93	Slonina *et al* (2014)	S2	2.93	2.98	3.35
6.862	0.561	0.065	7.5	Slonina *et al* (2014)	S2	7.5	7.85	9.03
8.71	0.75	0.119	1.11	Chaudhary *et al* (2014)	P2	1.11	1.13	1.29
8.71	1.02	0.061	4.02	Chaudhary *et al* (2014)	P2	4.02	4.50	5.16
8.71	1.29	0.041	7	Chaudhary *et al* (2014)	P2	7	7.33	8.42
8.71	1.7	0.079	11.9	Chaudhary *et al* (2014)	P2	11.9	12.61	14.63
8.71	1.87	0.074	18	Chaudhary *et al* (2014)	P2	18	19.62	23.12
8.71	2.43	0.057	22.6	Chaudhary *et al* (2014)	P2	22.6	25.23	30.29
8.71	0.66	0.117	1.2	Chaudhary *et al* (2014)	S2	1.2	1.250	1.42
8.71	0.89	0.075	2.6	Chaudhary *et al* (2014)	S2	2.6	2.64	3.04
8.71	1.15	0.047	4.5	Chaudhary *et al* (2014)	S2	4.5	4.76	5.43
8.71	1.36	0.037	13.4	Chaudhary *et al* (2014)	S2	13.4	14.24	16.56
8.71	1.61	0.023	21.7	Chaudhary *et al* (2014)	S2	21.7	22.7	26.97
8.71	2.01	0.011	25.9	Chaudhary *et al* (2014)	S2	25.9	26.7	32.2
10.115	1	0.041	2.02	Ando *et al* (2001)	S2	2.02	2.12	4.38
10.115	0.401	0.04	1.02	Ando *et al* (2001)	S2	1.05	1.06	1.14
11.192	0.62	0.041	2.35	Slonina *et al* (2014)	S2	2.25	2.29	2.54
11.192	0.686	0.035	2.93	Slonina *et al* (2014)	S2	2.93	2.98	3.35
11.192	0.772	0.026	7.5	Slonina *et al* (2014)	S2	7.5	7.85	9.03
12.379	0.814	0.056	7.9	Slonina *et al* (2014)	P2	7.24	7.44	8.55
13.063	0.63	0.062	7.9	Slonina *et al* (2014)	P2	7.24	7.44	8.55
16.805	0.855	0.04	7.9	Slonina *et al* (2014)	P2	7.24	7.44	8.55
19.381	0.861	0.038	2.35	Slonina *et al* (2014)	S2	2.25	2.29	2.54
19.381	0.894	0.031	2.93	Slonina *et al* (2014)	S2	2.93	2.98	3.35
19.381	1.096	0.01	7.5	Slonina *et al* (2014)	S2	7.5	7.85	9.03
27.81	0.364	0.029	1.85	Conti *et al* (1988)	P2	2.26	2.29	2.54
—	0.5	0	9.1	Belli *et al* (2000)	P2	9.43	9.73	11.24
—	0.49	0	21.4	Belli *et al* (2000)	P2	22.52	23.35	27.88
—	0.93	0	33	Belli *et al* (2000)	P2	36.68	38.17	49.60
—	0.55	0	7.7	Belli *et al* (2000)	P2	8.01	8.24	9.49
—	0.54	0	19.5	Belli *et al* (2000)	P2	20.78	21.57	25.57
—	0.52	0	29	Belli *et al* (2000)	P2	32.88	34.04	42.68
—	0.81	0	28.5	Antoccia *et al* (2009)	P2	36.20	37.63	48.62
—	0.01	0.138	1.85	Bettega *et al* (1979)	P2	2.26	2.29	2.54
—	0.156	0.169	3.9	Bettega *et al* (1979)	P2	4.12	4.20	4.77
—	0.515	0.082	5.5	Bettega *et al* (1979)	P2	5.57	5.70	6.52

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