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An imaging-based tumour growth and treatment response model: Investigating the effect of tumour oxygenation on radiation therapy response

Benjamin Titz¹ and **Robert Jeraj^{1,2,3}**

Benjamin Titz: titz@wisc.edu; Robert Jeraj:

¹ Department of Medical Physics, University of Wisconsin, Madison, WI, USA

² Department of Human Oncology, University of Wisconsin, Madison, WI, USA

³ Jožef Stefan Institute, Ljubljana, Slovenia

Abstract

A multiscale tumour simulation model employing cell-line-specific biological parameters and functional information derived from pre-therapy PET/CT imaging data was developed to investigate effects of different oxygenation levels on the response to radiation therapy. For each tumour voxel, stochastic simulations were performed to model cellular growth and therapeutic response. Model parameters were fitted to published preclinical experiments of head and neck squamous cell carcinoma (HNSCC). Using the obtained parameters, the model was applied to a human HNSCC case to investigate effects of different uniform and non-uniform oxygenation levels and results were compared for treatment efficacy. Simulations of the preclinical studies showed excellent agreement with published data and underlined the model's ability to quantitatively reproduce tumour behaviour within experimental uncertainties. When using a simplified transformation to derive non-uniform oxygenation levels from molecular imaging data, simulations of the clinical case showed heterogeneous tumour response and variability in radioresistance with decreasing oxygen levels. Once clinically validated, this model could be used to transform patient-specific data into voxel-based biological objectives for treatment planning and to investigate biologically optimized dose prescriptions.

Keywords

Tumour growth; tumour simulation; multiscale modelling; therapy response

1. Introduction

The reasons for treatment failures in radiation therapy differ with the type of tumour, but are often associated with oxygen-related effects such as hypoxia-induced radioresistance (Stone *et al* 1993, Brizel *et al* 1997, Hockel and Vaupel 2001) and increased metastatic propensity (Hill 1990, Hockel *et al* 1996). The presence of hypoxia has been demonstrated in squamous cell carcinomas of the head and neck (Nordsmark *et al* 1996), cervical cancer (Knocke *et al* 1999), melanoma (Rofstad and Maseide 1999), breast (Vaupel *et al* 1991), and prostate cancer (Movsas *et al* 1999). More importantly, hypoxia has been shown to predict an adverse treatment outcome (Hockel *et al* 1996, Brizel *et al* 1997, Nordsmark *et al* 2001). However, to this point,

the complex interplay between therapy-relevant properties such as hypoxia, clonogenic cell density, proliferation, and tumour treatment response is still not fully understood.

Computational tumour models can provide powerful tools to test the interplay of underlying biological processes and to identify important biological factors influencing therapeutic response. Radiobiological simulation models using specific tumour signatures were proposed for predicting therapy response (Wasserman and Acharya 1996, Stamatakos *et al* 2001, Borkenstein *et al* 2004), optimizing patient-specific therapies (Dionysiou *et al* 2004, Stamatakos *et al* 2006b), and as a “hypothesis generating tool” in cancer research (Sanga *et al* 2007).

Depending on which spatial scale the focus of interest resides on, computational models can be classified into three subcategories. In cellular or microscopic models (Stamatakos *et al* 2001, Harting *et al* 2007), each cell is modelled separately, describing cell-cell interactions and the behaviour of isolated cells. Due to computer memory limitations, these models are often limited to tumour sizes up to 1–2 mm³, depending on the number of cells simulated and the number of implemented biological processes. However, although a few exemplary microscopic models including pathological structural data (Pogue 2001) or the diffusion of metabolic waste products (Patel *et al* 2001) have been published, many publications lack fundamental biological input information. To that end, Capogrosso Sansone and coauthors (2001) concluded that the clinical measurement of the biological parameters used in simulations is crucial for a quantitative comparison between simulated results and actual clinical data.

In contrast to the microscopic models, macroscopic models do not keep track of single cells, but rather account for the tumour propagation by describing spatial and temporal changes in local tumour cell densities (Powathil *et al* 2007, Bondiau *et al* 2008). These models have been implemented based on reaction-diffusion equations and were extended to model locally alternating blood vessel densities, nutrient diffusion and cytotoxic therapies (Kohandel *et al* 2007, Powathil *et al* 2007).

Recently, interest has shifted away from exclusive growth modelling towards more complex modelling of angiogenesis, cytokinetic properties, and tumour response to different kinds of therapy. This gave rise to the third category of modelling approaches – so-called hybrid (Sanga *et al* 2007) or multiscale models (Dionysiou *et al* 2004, Jiang and Pjesivac-Grbovic 2005, Dionysiou *et al* 2006, Ribba *et al* 2006). The major advantage of these recent models is the capability of successfully combining mathematical models and engineering concepts with experimental data, enabling the incorporation of different physical scales into one model.

Unfortunately, detailed cell-line- or tumour-specific information provided by preclinical experiments or molecular imaging are frequently neglected. As a result, these models often cannot be validated or benchmarked against preclinical or clinical data. However, as Stewart and Li (2007) concluded in their work recently, there is a critical need for biologically realistic, predictive tumour simulation models, due to their potential ability to quantitatively connect empirical data to clinically observable outcome. This could improve the understanding of conflicting observations and help to unambiguously identify intratumoural mechanisms, which adversely affect therapy outcome.

In an optimal case, one would aim for combining biological parameters of a particular tumour with anatomical and functional information from clinical imaging data into a multiscale approach to “feed” information both to a cellular level and a higher order level, thereby simulating the tumour as a functional entity. Molecular imaging techniques such as positron emission tomography (PET) allow for the non-invasive visualization of spatially heterogeneous physiological information. They enable imaging of therapy-relevant parameters

such as proliferative activity, tissue oxygenation, and angiogenic activity, and were proposed to aid the individualization of cancer treatments (Rasey *et al* 1996, Chapman *et al* 2001, Mankoff and Bellon 2001). Once successfully validated, a tumour simulation model accounting for these physiological data could be then utilized to generate and optimize treatment plans under consideration of patient-, tumour-, and organ-specific biological parameters.

The purpose of this work was to design and implement a computational model which is able to incorporate patient-specific anatomical and biological information in addition to tumour-specific biological parameters. To this end, we developed a multiscale tumour model employing cell-line-specific input variables and cell kinetic parameters derived from pre-therapy PET/CT imaging data. We currently limit our imaging-based input parameters to information on cellular proliferation and tumour oxygenation. Additional model parameters are fitted to published *in vitro* and *in vivo* models of head and neck squamous cell carcinoma (HNSCC) cells. Using the obtained set of parameters, the model is applied to a human HNSCC case to investigate effects of different uniform and non-uniform oxygenation levels. Results are compared for treatment efficacy under various hypoxic conditions.

2. Tumour growth and treatment response model

Our tumour simulation model consists of two parts, shown in figure 1: a) a microscopic cellular layer at which simulations based on input parameters from preclinical studies and clinical imaging are carried out, and b) a macroscopic tissue layer which is available in voxel space to allow for comparison to clinical imaging data. Preferably, all input parameters used in the model should be patient- or at least tumour-specific. However, if patient-specific data are not available directly, tumour cell-line-specific data can be used in the model instead. General input parameters, which are assumed to be valid independently of the type of tumour (for example the tumour cell size), constitute the framework of the simulation model and remain constant.

In this work, we focused on two molecular markers used in PET imaging to assess patient-specific input parameters: 3'-Deoxy-3'-[^{18}F]fluorothymidine ([^{18}F]FLT), a cellular proliferation surrogate (Kubota *et al* 1992, Van Waarde *et al* 2004), and Copper(II) diacetyl-di(*N*⁴-methylthiosemicarbazone) ([^{61}Cu]Cu-ATSM), a marker of cellular hypoxia first introduced by Fujibayashi *et al* (1997).

At the cellular level, the content of each tumour voxel is simulated cell-by-cell based on cell-line specific parameters, such as the characteristic cell cycle time and cell cycle phase-dependent radiosensitivity values. The model accounts for biological processes such as quiescence, apoptosis and radiation-induced necrosis; cellular proliferation is treated in a semi-probabilistic manner, which is discussed below. Changes resulting from simulations at the microscopic level are then passed on to the tissue level, where biological information is updated accordingly.

2.1. Cellular layer

Cellular growth concept—In contrast to other cellular models (Kocher and Treuer 1995, Stamatakis *et al* 2001, Borkenstein *et al* 2004, Dionysiou *et al* 2004), which are based on cubic grids with each voxel representing a normal tissue or cancer cell, our model is purely stochastic on the cellular scale. As a result, computing time is minimized by running simulations at the microscopic level in 1D space.

The currently implemented biological parameters include the cell cycle time (T_c) to account for cell cycle checkpoints and cell cycle phase dependant parameters such as the intrinsic radiosensitivity, which determines the cell's survival probability following irradiation and

varies within the mitotic cycle (Hall 2000). At the onset of a simulation, each tumour voxel is assumed to contain N cells (see table 1). The oxygenation status and the proliferative potential for each voxel are derived from [^{18}F]FLT and [^{61}Cu]Cu-ATSM PET scans (as described later). Apoptosis and cellular lysis following cell death are treated in a simplified manner, assuming that cell masses are broken up and removed from the tumour within 5 days after cell death.

As the computer simulation proceeds, the age of each cell is incremented in predetermined time steps. Typically, 1-hour time steps are sufficient since short-lived cellular processes such as lysis and mitosis occur on a time scale of several hours. The cell cycle time is assigned to every cell either at the onset of the simulation or whenever a new cell is “born” during the simulation.

Generally, there are two major checkpoints within the eukaryotic cell cycle: a) the G_1/S checkpoint which occurs at the end of the first gap in activity (G_1) before the cell enters the S (DNA synthesis) phase and b) the G_2/M checkpoint which occurs before the cell enters the mitotic (cell division) phase (Hall 2000). Both checkpoints represent a decision making process whether the cell should divide or not, depending on environmental conditions. Another important function of the checkpoints is to assess DNA damage (such as radiation induced damage) and decide if repairs can be made or not.

In addition to these two checkpoints, another checkpoint within the G_1 phase has been implemented in our model to account for a non-dividing phase outside the cell cycle: If the cell experiences a severe lack of oxygen, it enters a state of quiescence, the G_0 phase, for a predefined time frame. In the implemented model shown in figure 2, a quiescent cell undergoes apoptosis after failing to reoxygenate within the maximum allowed time frame. Reoxygenation is a phenomenon in which hypoxic cells become re-exposed to oxygen by coming into closer proximity to capillaries (Kallman and Dorie 1986). In general, reoxygenation occurs after chronic hypoxia. Acute (transient) hypoxia is not taken into account in our simulations due to its unpredictable and short-lived nature (Hockel and Vaupel 2001). Upon reoxygenation, a cell can re-enter the cell cycle, as illustrated in figure 2.

Above the necrosis threshold of 1 mmHg oxygen partial pressure (pO_2) (De Los Santos and Thomas 2007) and up to a vascular pO_2 value of 60 mmHg, we determine the probability of cell division by calculating an oxygen-dependant, Gompertzian probability distribution similar to the oxyhaemoglobin dissociation curve (Aberman *et al* 1973), which describes the relationship between available oxygen and the amount of oxygen carried by haemoglobin. The probability P for cell division depends on the cell's current oxygenation status such that

$$P(pO_2) = C \cdot \exp \{ -\exp[-B \cdot (pO_2 - M)] \}. \quad (1)$$

Here, C is the upper asymptote, B is the growth rate and M is the inflection point (the pO_2 value at the point of maximum incline). Based on this relationship, a cell in our model can undergo cell division if a sampled random number is less than or equal to the probability $P(pO_2)$.

Modelling response to radiation therapy—A rather simple model for cell survival following radiation therapy that is widely used is the linear quadratic model (LQM). The model underlies the linear-quadratic formalism for DNA damage (Withers 1975, Brenner *et al* 1991) and is primarily based on the stem cell principle (Baguley 2006). The latter is justified with the observation that tumour cells have the potential for limitless replication (Hanahan and Weinberg 2000) and are often continuously proliferating, but non-functional cells (Lohr *et al* 1993).

In our model, we assume that analogous to the fraction of cells surviving an irradiation event and based on the LQM, a normoxic cell survives if a random number drawn from a uniform distribution is less or equal than its individual survival probability P determined by

$$P(D) = \exp \left[-\alpha D \cdot \left(1 + \frac{D}{\alpha/\beta} \right) \right]. \quad (2)$$

In here, α and β are the radiosensitivity coefficients for linear (αD) and quadratic contributions (βD^2) of the absorbed dose D to the induced damage. The radiosensitivity can be calculated by using information on the current cell cycle phase (Ling *et al* 1995) and the oxygenation status of a cell. Since the oxygen effect, which can be quantified in the LQ model by the oxygen enhancement ratio (OER), shows significant contributions at oxygen levels of 20 mmHg or less (Hall 2000), an additional oxygen dependant modification factor (OMF) is introduced, so that the survival probability for a hypoxic cell can be calculated from

$$P(D, pO_2) = \exp \left[-\alpha D \cdot OMF(pO_2) \cdot \left(1 + \frac{D \cdot OMF(pO_2)}{\alpha/\beta} \right) \right]. \quad (3)$$

Here, the OMF is given by the OER at the current pO_2 normalized by the maximum OER. By applying this equation, we are able to calculate the post-irradiation survival probability for every single cell using individually sampled, cell cycle phase-dependent radiosensitivity values at discrete time points. This feature is of crucial importance since cell growth and cell death are modelled independently, but occur simultaneously by nature. Accordingly, the OER has a significant impact on the cell survival fraction. Alper and Howard-Flanders (1956) established a relationship between the observed increase in radiosensitivity with increasing oxygenation levels. Since then, the oxygen dependence of the LQM has been studied extensively, e.g. by Wouters and Brown (1997) and Buffa *et al* (2001).

In contrast to other simulation models (Stamatakis *et al* 2001, Borkenstein *et al* 2004), which do not account for or simplify this relationship, we model the OER as a continuous function of the pO_2 according to a relation Alper (1979) derived based on Michaelis-Menten kinetics. In this model, the OER is described as the ratio of doses necessary in order to achieve the same cell survival under conditions of different oxygenation so that

$$OER(pO_2) = \frac{D_{anoxic}}{D_{oxic}} = \frac{m \cdot pO_2 + K}{pO_2 + K}. \quad (4)$$

Here, D_{anoxic} and D_{oxic} represent the radiation doses needed for the same cell kill under anoxic and oxic conditions, m is the maximum ratio, and K is the pO_2 at half the increase from 1 to m . The resulting continuous function can be sampled for every pO_2 value and is shown in figure 4, below.

Since characteristic half times for damage repair are on the order of a few hours, we assume complete repair and full recovery between fractions. The fragmentation and resorption of dead cells occurs within a given lysis time T_{lys} (see table 1).

2.2. Model input parameters

General model input parameters—General input parameters are assumed to be universally valid and do not change with the type of tumour, the cell-line, or with a different patient. Examples for general parameters are the tumour cell volume (which is assumed to be constant across all tumour cells), the maximum *OER*, and the oxygen threshold at which cells become necrotic, as shown in table 1.

Input parameters from literature and preclinical tumour models—In order to tune the simulation model to a specific tumour type, relevant parameters from literature and preclinical *in vivo* experiments are incorporated into the model. In our specific case, radiosensitivity coefficients, the cell cycle time, and initial cell cycle phase distribution, are adapted to published values (summarized in table 1) for a head and neck (H&N) tumour cell line. Missing parameters are then determined by comparing simulated tumour growth and response curves to a published preclinical experiment, as described later.

Input parameters from clinical imaging—The CT data are used solely to extract the initial tumour anatomy in form of a tumour mask, which is then used to extract tumour voxels from the PET scans as described in the image processing section.

The uptake of the PET tracer [^{18}F]FLT is used as an index of cellular proliferation (Shields *et al* 1998). Ideally, one would derive the number of proliferating cells contained within each tumour voxel directly from PET imaging data. This number would then serve as the initial population of actively proliferating tumour cells at the onset of the simulation. However, this is not feasible since the tracer uptake of a single proliferative cell is not quantifiable and the standardized uptake value (SUV) of a voxel represents an averaged value over all cells contained within this voxel. As an approximation, we assume direct proportionality between the number of proliferative cells within a voxel and the measured SUV value for this particular voxel. The number of proliferative cells is then calculated by assuming 10^6 cells per mm^3 (Steel 2002) and multiplying this value by the SUV of the voxel. After each simulated time step, changes in the cell number due to cellular growth or therapeutic response are accounted for by multiplying the initial SUV by the relative increase or decrease of proliferating cells.

In order to predict therapeutic response, it is desirable to obtain the $p\text{O}_2$ distribution within the tumour for each patient. [^{61}Cu]Cu-ATSM has a high selectivity for oxygen deficient cells (Fujibayashi *et al* 1997) and has been used as a PET tracer to delineate ischemic and hypoxic myocardial tissue (Lewis *et al* 2002) as well as to identify hypoxic subvolumes within tumours (Chao *et al* 2001). The uptake was shown to be strongly dependent on the local oxygen concentration (Lewis *et al* 2001). Clinical studies using [^{61}Cu]Cu-ATSM in patients with cervical cancer (Dehdashti *et al* 2003a) and lung cancer (Dehdashti *et al* 2003b) showed that uptake values provide information that was predictive of therapeutic response.

However, to this point, the exact quantitative relationship between the SUV of [^{61}Cu]Cu-ATSM and the local tissue oxygenation is not known. As an approximation, we chose a sigmoid relationship (shown in figure 4) justified by findings of Lewis and co-authors (1999). In addition, different uniform oxygenation levels that were independent of the SUV of [^{61}Cu]Cu-ATSM were investigated.

A summary of all input parameters is given in table 1.

2.3. Tissue layer

On the macroscopic tissue level, data are contained in voxels, allowing for comparisons between the simulated radiation response and actual follow-up PET/CT scans after

radiotherapy. Biological information contained within the PET images is updated iteratively with the results of the underlying simulations at the microscopic level. Thus, each voxel represents averaged values of the metabolic parameters of all cells contained within.

Currently, the model is non-deformable, which represents a limitation since only tumours with minimal morphological changes can be simulated.

3. Materials and Methods

3.1. Clinical imaging protocol and image processing

A total of three pre-therapy PET/CT scans of a human H&N patient were conducted on a GE Discovery LS PET/CT scanner (General Electric, Waukesha, WI). In each case, the patient was positioned and immobilized supine to allow for accurate image coregistration. PET imaging began with an [^{18}F]FDG scan in order to detect alterations in glucose metabolism. After intravenous injection of approximately 15 mCi of [^{18}F]FDG ($t_{1/2} = 110$ min), PET emission data were acquired for 30 min in a static mode. In addition, a [^{61}Cu]Cu-ATSM ($t_{1/2} = 3.33$ hrs, injected activity 4 mCi) PET scan and a [^{18}F]FLT ($t_{1/2} = 109.77$ min, injected activity 6.55 mCi) PET scan were conducted. In the [^{61}Cu]Cu-ATSM case, a static 30-minute PET scan was performed 3 hrs post-injection. A dynamic 90-minute PET scan was performed immediately following the injection of [^{18}F]FLT. CT attenuation-corrected PET images were reconstructed on the clinical scanner using an OSEM2D algorithm with 2 iterations and a voxel size of $0.391 \times 0.391 \times 0.425$ cm (0.065 cm³). Frames of the last 30 minutes of the dynamic [^{18}F]FLT PET scan were averaged to obtain SUVs representing a temporal mean. All studies were carried out in accordance with the IRB approved research protocols.

The data were then imported into AMIRA (Mercury Computer Systems, Inc.) for visualization and tumour segmentation and registered with the *AffineRegistration* module using the extensive direction optimizer. The initial tumour anatomy was acquired by manually segmenting the tumour on the CT data set. In areas of low contrast between the tumour and normal tissue, the segmentation process was aided by the [^{18}F]FDG PET data to distinguish between normal and cancerous tissue. The tumour segmentation was converted into a binary tumour mask which was then multiplied with the registered [^{18}F]FLT and [^{61}Cu]Cu-ATSM scans in order to eliminate tracer uptake in areas other than the tumour. Relevant PET data were imported into Matlab 7.0 (The MathWorks, Inc) to run simulations and then transferred back to AMIRA for visualization.

3.2. Tuning and application of the model

To test the effect of oxygen on therapy response at the underlying cellular level, we simulated radiation response of a single voxel under oxic conditions, hypoxic conditions, and under initial hypoxia followed by reoxygenation during the treatment regime.

Dynamics observable within the cell cycle, such as the redistribution of the cell cycle phases after an irradiation event, were validated in a second simulation by comparing the model predictions to data published by Johnson *et al* (1997). The simulated cell population was assumed to be freely cycling (not synchronized), irradiated with a single dose of 2 Gy and then followed for 50 hrs while monitoring the fractional distribution of the different cell cycle phases.

After evaluating the implemented model on the cellular scale, the model was compared to preclinical experiments in order to test growth kinetics and spatiotemporal behaviour of the tumour. To that end, the model was benchmarked against a preclinical experiment (Huang and Harari 2000) using the HNSCC-1 cell line, which included unrestricted growth as well as tumour response following radiation therapy. The cell cycle time was adapted to a value for

HNSCC-1 cells published by Easty *et al* (1981) and the average oxygen level ($pO_{2\text{ avg}}$) as well as the cell lysis time (T_{lys}) were adjusted to match the growth curve. The same parameters were then used to simulate radiation response to the fractionation pattern used in the experiments.

Finally, using the parameters obtained from comparisons to *in vitro* and animal data, the model was applied to a human HNSCC case. In this clinical case, however, the voxel-specific oxygenation and proliferation levels derived from the PET/CT images were included in the simulations. By applying the clinical fractionation pattern, therapeutic response was simulated based on different uniform and nonuniform oxygenation levels and compared for treatment efficacy.

4. Results

4.1. Cellular layer simulations

Effect of oxygen on therapy response—The resulting effects of different average oxygenation levels on the response to radiation therapy are shown in figure 5. In both the oxic and the hypoxic case, the oxygenation level was held constant.

Simulated radiation responses show the ability of the model to account for reoxygenation occurring during the course of the irradiation process. Hypoxic cells required significantly higher doses in order to achieve the same survival fraction. However, if dead cells were removed, reoxygenation could occur and radiosensitivity increased with the increasing average oxygenation level, resulting in a faster cell kill. The underlying condition for this simulation was a constant supply of oxygen while the total number of cells decreased. The rate at which this reoxygenation process occurs is governed by the removal time for dead cells.

Redistribution following radiation therapy—The initial cell cycle phase distribution was assumed to be 60 % in G_1 , 25 % in S, and 15 % in G_2/M , which is in the range of values Huang *et al* (2002) found for HNSCC-1 cells. Simulation results of an oxic (60 mmHg) and a hypoxic case (2 mmHg) are shown in figure 6.

Simulation results exhibit good qualitative and quantitative agreement with the experiments. In each cell cycle phase, the model was able to capture the key characteristics. Since the results shown only account for the fractional distribution within the cell cycle rather than the remaining fraction of cells, the curves for cells under oxic and hypoxic conditions differ only slightly. However, the hypoxic curve features a ringing effect, which is due to the slower proliferation rate of the hypoxic cell culture.

4.2. Tissue layer simulations

The results of the experimental comparison with the data published by Huang and Harari (2000) are shown in figure 7.

The comparison shows that the model was able to quantitatively and qualitatively reproduce macroscopic tumour behaviour such as tumour shrinkage and regrowth after therapy within experimental uncertainties. Similar to the experiment by Huang and Harari (2000), administration of radiation therapy caused a decrease in the tumour growth rate, shrinkage of the tumour and a delay in tumour growth. The delay of the tumour shrinkage relative to time of therapy is partially due to the lysis time, T_{lys} , which is required to completely remove dead cells (in contrast to the results of the next section, tumour volume was calculated based on the tumour cell volume, V_c , and the total number of remaining viable cells).

4.3. Application of the model to clinical data

For this simulation, additional patient-specific information on proliferation and pO_2 levels was extracted from pre-therapy PET/CT images (see figure 8, bottom left) and included in the simulations. The oxygen levels for the simulations were a) non-uniform, derived from the hypoxia PET scan, b) 2.5 mmHg, a commonly used threshold for hypoxia, c) 5 mmHg and d) 9 mmHg, a median pO_2 value found for H&N tumours (Nordsmark *et al* 2005). Simulations were conducted using a clinical fractionation pattern of 2 Gy/day delivered on weekdays for 3 weeks. Results are shown in figure 8.

In general, treatment efficacy was reduced with lower oxygen levels. Accounting for the inhomogeneous oxygen distribution, which was derived from the $[^{61}\text{Cu}]\text{Cu}$ -ATSM scan, resulted in inhomogeneous tumour response with the highest fraction of surviving cells localized in the area of high tumour hypoxia, as shown in figure 8 (top right). This suggests that application of uniform dose distributions without accounting for tumour heterogeneity results in a higher survival fraction within hypoxic subpopulations.

5. Discussion

The multiscale tumour simulation model presented in this work is able to realistically reproduce biological phenomena. Model predictions of therapeutic response could be verified against *in vitro* and preclinical data. Simulating therapy response on a cellular scale (figure 5) demonstrated the ability of the model to successfully account for reoxygenation during radiation therapy. The speed at which reoxygenation occurs in this process will partially depend on T_{lys} , the time required for the lysis of dead cells. If applied to simulations on the tissue scale, a second factor influencing the rate of reoxygenation is the composition of a voxel, that is, the percentages of actively proliferating cells and quiescent cells within the voxel.

When simulating the cell cycle redistribution process of cultured cells following a single irradiation event, the comparison of the simulated redistribution curves to data published by Johnson *et al* (1997) demonstrates that the model predictions were able to capture the key characteristics in each phase (see figure 6). Interestingly, simulated response curves for hypoxic conditions feature a slight ringing effect due to a longer potential doubling time compared to cells under oxic conditions. Accordingly, hypoxic cells remain in a state of radiation-induced synchronization for a longer time, resulting in a longer time frame required for the redistribution towards the initial distribution among the different cell cycle phases. These results indicate the ability of the implemented cell cycle model to reproduce biologically realistic cell cycle dynamics and responses to radiation therapy. Cell cycle dynamics, however, might differ with the type of tumour or the cell line. Accordingly, the use of reliable biological parameters for the simulations is crucial and values should be adapted to the adequate cell line whenever data are available from literature and/or experiments. In order to reproduce the experiment conducted by Johnson *et al* (1997), the initial distribution of the cell population within the cell cycle should be existent in a biologically realistic manner. For this, cells must not be synchronized at the onset of the simulation, but rather need to be arranged in an exponential distribution through the cell cycle (Steel 2002) at the time point of the simulated irradiation event. However, even if the cell population gets synchronized, the simulation model is able to reproduce a biologically realistic redistribution process, as shown in figure 6.

The comparison of simulated results and preclinical data (figure 7) shows that macroscopic tumour behaviour could be reproduced for both the unrestricted growth case as well as the response to radiation therapy case. It should be stressed that values of the implemented biological parameters were identical in both cases.

The central question stemming from these comparisons with preclinical data and concerning the application of the model to clinical data is in how far results acquired by *in vitro* and/or preclinical experiments can be translated directly into clinical models. In the past, for example, both the LQM and the OER paradigm were shown to be valid for *in vitro* studies but failed to prove their one-to-one applicability to *in vivo* cases (Bentzen *et al* 1990, Roberts and Hendry 1998). Nonetheless, the LQM is included in concepts such as the biologically effective dose, which is commonly used in clinical practice (Fowler 2001). This is mainly due to the fact that certain parameters or processes currently can only be measured *in vitro* and accordingly, no *in vivo* data are available. For example, it is not ethically feasible to record a tumour growth curve in a patient. At the same time, it is important to benchmark simulation models for cases of unrestricted growth (without therapy) to verify simulated growth behaviour of the tumour on days without administration of therapy (such as weekends, for example).

The alternative is to limit simulations exclusively to clinical scenarios. Other models (Dionysiou *et al* 2004, Stamatakos *et al* 2006a, Powathil *et al* 2007, Bondiau *et al* 2008), for example, have been applied to clinical data without investigating and validating underlying cell cycle dynamics. The shortcoming of such an approach is that the underlying biological processes can hardly be tuned and verified. Thus, a quantitative comparison between model predictions and experimental data constitute a crucial step before applying the model to any higher scale, such as patient data on a voxel scale.

In our pilot simulations based upon clinical data (figure 8), treatment efficacy was reduced with lower oxygen levels when accounting for spatially heterogeneous biological information derived from pre-therapy [⁶¹Cu]Cu-ATSM PET/CT images. Although the retention of [⁶¹Cu]Cu-ATSM has been shown to correlate with tissue *pO*₂ *in vivo* (Lewis *et al* 2001), the radiation resistance derived from PET data will ultimately depend upon the chosen transformation to translate measured SUVs into clinically relevant hypoxia levels. Accordingly, the investigation of the quantitative relationship between *pO*₂ and [⁶¹Cu]Cu-ATSM uptake is of great importance. This might also provide the opportunity to validate the translation of hypoxia PET data into a clinical OER and to estimate voxel-based, clinical radiosensitivity coefficients (α and β of the LQM). Alternatively, other PET tracers such as [¹⁸F]FMISO (Rasey *et al* 1996) or [¹⁸F]FAZA (Piert *et al* 2005) could be used to assess tumour hypoxia.

Simulation models such as the one presented in this work provide virtual environments to test various hypotheses. The main objective, however, is the clinical validation of the model predictions. A potential way to verify this model on a clinical imaging scale is to acquire multiple PET/CT scans before, during and after the administration of therapy, using the pre-therapy data as model input data and comparing the model predictions to the follow-up scans after therapy.

In order to make this feasible, however, future work will also require the addition of a deformation module to account for physical tumour growth and shrinkage, since the current model includes cell death and cell lysis but no macroscopic tumour deformation yet. Allowing for tumour deformation during an anti-cancer therapy will ultimately allow for an evaluative comparison with follow-up PET/CT scans, even if they are conducted at time points later in the treatment schedule (when deformations usually occur), and enable further tuning and benchmarking of the model against clinical cases.

After successful clinical verification, this model has the potential to become a valuable tool in the process of transforming patient-specific tumour information into voxel-based parameters for outcome modelling. It could then be used to investigate biologically based non-uniform dose prescriptions and help identify a biologically optimized treatment regime, such as in hypoxia-based dose painting.

6. Conclusion

The multiscale simulation model presented in this work is able to simulate tumour growth as well as radiation therapy response while allowing for the integration of and comparison with molecular imaging data. Model predictions of response to radiation therapy could be verified against *in vitro* and preclinical data.

One of the benefits of the chosen hybrid approach is that input parameters can be acquired via cell-line-specific studies and tumour-specific molecular imaging data depicting unique biological characteristics. In addition, the model can be easily extended to simulate effects of chemotherapy, combination therapies, and effects of non-uniform dose distributions. Future work will include the addition of a deformation algorithm in order to account for anisotropic changes in the macroscopic tumour geometry.

Once successfully validated against clinical cases, this model could be used to investigate biologically based non-uniform dose prescriptions based on patient-specific radiosensitivity parameters and help identify a biologically optimized treatment regime for use in biologically guided radiation therapy by providing biological objectives.

Successful biological modelling and the prediction of patient-specific therapeutic response have the potential to improve the efficacy of anti-cancer therapies and might accelerate the process of establishing biologically guided radiation therapy in the clinic.

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Figure 1.

Flowchart of the developed multiscale tumour simulation model. At the cellular layer, each voxel is simulated based on cell-line-specific information, which is complemented by patient-specific input parameters from clinical imaging. Thus, the modelled PET images 1 and 2 represent simulation results based on the clinical PET images 1 and 2, and can be validated against follow-up PET/CT scans.

Figure 2. Schematic illustration of the implemented cell cycle. An additional checkpoint (denoted by red arrowhead) was introduced to determine whether the cell enters the G₀-phase. If the cell remains quiescent for a certain time, it subsequently undergoes apoptosis.

Figure 3.

Dependence of the relative radiosensitivity (expressed through the OER) and the OMF as a function of the pO_2 . In the simulations, a maximum ratio m of 3 and a K value of 3 mmHg (Toma-Dasu *et al* 2001, Nilsson 2004) were used, yielding a radiosensitivity curve in excellent agreement with the one given by Hall (2000) and experimental values found for sarcomas (Nash *et al* 1974).

Figure 4. Illustration of the investigated sigmoid relationship between the oxygen partial pressure (pO_2) and the SUV of [^{61}Cu]Cu-ATSM measured *in vivo*.

Figure 5.

Therapy response as a function of different average oxygenation levels. Simulations were carried out on a single voxel containing 10^6 cells using a linear radiosensitivity coefficient of 0.3 Gy^{-1} and a α/β -ratio of 10 Gy (Schwachhoefer *et al* 1990). The dose was administered continuously with a fractionation of 2 Gy per day.

Figure 6. Comparison of simulated redistribution curves and data published by Johnson *et al* (1997). Note the ringing of the hypoxic curve, which is due to the fact that the redistribution process occurs at a slower rate compared to the one of the oxic curve.

Figure 7. Benchmark of the tumour model against a published HNSCC-1 cell line experiment. Values for the control cell line (black) and the irradiated cell line (red) were adapted from the original work^c. Yellow arrowheads represent the days on which radiation therapy (XRT) was administered in the actual experiments as well as in the simulation.

^cFor the tumour volume measurement of the actual irradiated cell line on day 56, the original publication did not include an error bar.

Figure 8.

Clinical simulation input data and simulated therapeutic response under different oxygenation levels. **Left:** Axial slice of a pre-therapy $[^{18}\text{F}]\text{FLT}$ PET/CT denoting the tumour location. Prior to the first fraction, the patient was scanned to assess levels of proliferation and hypoxia within the tumour (shown bottom left). **Right:** Time course of the simulated therapeutic response as a function of a non-uniform (top row) and different uniform oxygenation levels (rows 2, 3, and 4). The remaining fractions (cell distribution after three weeks XRT normalized to the initial distribution) are shown in the right column. Note the different colour bars for different time points.

Table 1

Example of input parameters used in this work.

Model parameter	Symbol	Value
<i>General (universally valid) input:</i>		
Cells per voxel	N	$10^6/\text{mm}^3$ (Steel 2002)
Assumed cell volume	V_c	$10^3 \mu\text{m}^3$ (Steel 2002)
Maximum OER value	m	3 (Nash <i>et al</i> 1974, Hall 2000) ^a
pO_2 at OER = $(m+1)/2$	K	3 mmHg (Hall 2000, Toma-Dasu <i>et al</i> 2001)
Necrosis threshold	$pO_{2 \text{ necr}}$	1 mmHg (De Los Santos and Thomas 2007)
<i>Cell-line-specific input:</i>		
Average cell cycle time	T_c	36 hrs (Easty <i>et al</i> 1981)
Radiosensitivity coefficient α	α	0.3 Gy^{-1} (Schwachhoefer <i>et al</i> 1990)
Radiosensitivity coefficient β	β	0.03 Gy^{-2} (given through α and α/β)
Alpha-beta-ratio	α/β	10 Gy (Schwachhoefer <i>et al</i> 1990)
Fraction of cells in G1 phase	F_{G1}	60 % (Johnson <i>et al</i> 1997, Huang <i>et al</i> 2002)
Fraction of cells in S phase	F_S	25 % (Johnson <i>et al</i> 1997, Huang <i>et al</i> 2002)
Fraction of cells in G2/M phase	$F_{G2/M}$	15 % (Johnson <i>et al</i> 1997, Huang <i>et al</i> 2002)
Average tumour oxygenation	$pO_{2 \text{ avg}}$	16.7 mmHg ^b
Time for lysis and complete removal	T_{lys}	5 days ^b
<i>Patient-specific input:</i>		
Proliferating cells per voxel	N_{pc}	derived from [^{18}F]FLT PET scan
Oxygen partial pressure of voxel	pO_2	derived from [^{61}Cu]Cu-ATSM PET scan

^aThis represents a theoretical value. Inserting a vascular pO_2 into equation (4) leads to a maximum OER of 2.9.

^bValues were determined from a simulation based on results of Huang and Harari (2000), as described later.