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A Three-Dimensional Tumour Growth Model for a Radiation Therapy Process Simulation

by

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B. Sc. (National University of Defense Technology, 1991)

A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfillment of
the requirements for the degree of

Master of Science

Ottawa-Carleton Institute for Physics

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the Faculty of Graduate Studies and Research
acceptance of the thesis

**A Three-Dimensional Tumour Growth Model
for a Radiation Therapy Process Simulation**

Submitted by

Zhanrong Gao, B.Sc.

In partial fulfillment of the requirements
For the degree of Master of Science

Chair, Department of Physics

Thesis Supervisor

date Sept 10, 2001

Carleton University

ABSTRACT

This thesis describes a **Double kinetic Gompertzian-Exponential (DGE)** model, which is designed to be a spatial and temporal model to simulate tumour growth for clinical research applications. This unique model can provide a link between clinical spatial information and a radiobiological cell kill model for our overall radiotherapy process model, which is aimed at the investigation of the impact of technological change on the patient outcome.

The DGE tumour growth model, which was first suggested by our group, is extended from the well-accepted Gompertzian-Exponential growth model. Our modification mainly addresses local and systemic feedback inhibition. We assume that local feedback is at the voxel level and that systemic feedback is associated with patient tumour burden. Feedback at the voxel level can be considered as the condensation of all local biological factors which inhibit tumour growth. Both the systemic feedback and local feedback are described by the Gompertzian-Exponential growth model. Furthermore, the model is extended to a spatial character with the assumption that cells in one voxel can spread to the adjacent voxels with a certain probability. The spread is driven by the tumour cell concentration gradient.

A series of tests have been performed and three sets of experimental growth data including two spheroid data sets and one virtual untreated brain tumour have been used to validate this model. The results showed that this model could simulate not only symmetric tumours such as found at spheroid and solid tumour but also the asymmetric growth such as at tissue interfaces, as well as local regional nodal involvement. This model can predict behaviour similar to that seen in the clinic and laboratory. Since this model is a voxel based tumour growth model, it provides an opportunity to link with the spatial tumour information acquired from standard imaging to investigate radiotherapy.

This DGE model is a semi-empirical model. It reflects the characteristics of the entire population of a specific tumour, and does not provide a basis for understanding or predicting individual tumour behaviour.

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LIST OF ABBREVIATIONS

AAPM	America Association of Physicists in Medicine
CA	Cellular Automaton
CTV	Clinical Tumor Volume
DGE	Double kinetics Gompertzian Exponential
GBM	GlioBlastoma Multiforme
GTV	Gross Tumour Volume
HCM	Homeostatic Control Mechanism
IAEA	International Atomic Energy Agency
ICRP	International Committee of Radiological Protection
ICRU	International Commission on Radiation Units and measurements
MLC	MultiLeaf Collimator
NTCP	Normal Tissue Complication Probability
ODE	Ordinary Differential Equation
ORCC	Ottawa Regional Cancer Center
ROSS	Radiation Oncology Simulation System
T_{pot}	Potential Doubling Time
TBI	Total Body Irradiation
TCD	Tumour Cell Density
TCP	Tumour Control Probability
TG	Tumour Growth
TLI	Thymidine Labeling Index
TAF	Tumour Angiogenesis Factor
VB	Microsoft® Visual Basic
VC	Microsoft® Visual C++
VR	Virtual Radiotherapy

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LIST OF SYMBOLS

$\rho(x, y, z, t)$	: tumour cell density at coordinate (x,y,z) at time t
T_d	: cycling time for cells
G_0	: quiescence state
$n(r, t)$	: tumour cell density at radius r at time t
D_c	: diffusion coefficient
N_0	: the initial number of tumour cells
T_{pot}	: potential doubling time of tumour cells
K_b	: birth rate of tumour cells
K_l	: loss rate of tumour cells
N_{max}	: Maximum number of cells or tumour burden
N_g	: transform point from Exponential growth to Gompertzian growth for entire tumour
N	: the current number of tumour cells
$x_{i,j,k}, y_{i,j,k}, z_{i,j,k}$	: the coordinate of the center of voxel (i,j,k)
$\Delta N_{i,j,k}$	: the number of new generated tumour cells in voxel (i,j,k)
k_{global}	: global cell loss rate
$k_{l(i,j,k)}$	: local cell loss rate
$N_{i,j,k}$	: number of tumour
$C_{i,j,k}$	: the capacity to accept cells from other voxels
$N_{max\ voxel}$	: Maximum cell number in a voxel
N_{gvoxel}	: transform point from exponential growth to Gompertzian growth in local voxel
$V_{l,m,n}$	: the geometry correction factor
k^{out}	: The diffusion coefficient of tumour cells to move out
k^{in}	: the diffusion coefficient to accept tumour cells from other voxels
$\zeta_{i,j,k}$	: the number of tumour cells migrated out of current voxel
$\delta_{i,j,k}$	: the net cell growth in voxel (i,j,k)
$\phi_{i,j,k}$	: the number of tumour cells move in from adjacent voxels
ξ_l	: random number
$\eta_{i,j,k}$	: number of tumour cells in voxel (i,j,k)
$\sigma_{i,j,k}$	: statistics error
Δt	: time increment (hours)

N_l	: number of calculation loop
$P_{i,j,k}^{nodal}$	: probability to occur a nodal involvement in voxel (i,j,k)
$k_{i,j,k}^{nodal}$	: the coefficient to occur nodal involvement in voxel (i,j,k)
r_n	: radius of necrosis in a spheroid (mm)
r_t	: radius of tumour (mm)
f_n	: fraction of necrosis in a spheroid
V_1, V_2	: volume of tumour at time t_1 and t_2
$\varpi_{sf}(x, y, z)$	: survival fraction in coordinate (x,y,z)
$D(x, y, z)$	: dose delivered at coordinate (x,y,z)
α, β	: two parameters of the L-Q model
VCP_j	: volume control probability at voxel j.
T_{vi}	: time when tumour can be diagnosed
T_{re}	: time when tumour start to treat
T_{tr}	: time when tumour finish treat
N_f	: critical number of cells in a tumour, below it, which means tumour free.

CHAPTER 1

INTRODUCTION

1.0 Overview of This Chapter

This chapter briefly reviews the history of radiotherapy, the role of radiotherapy in the improvement in cancer treatment over the last thirty years and the challenges facing this field today. Analysis of the correlation among technological improvement, cost escalation and tumour control rates over the past three decades suggests that further improvements in cancer control rates due to radiotherapy must come from modifications to the entire radiotherapy process, not just the accuracy of dose delivery. This is the focus of the work being performed by the ORCC research group, that is the development of a model to predict the effect of process change on patient outcome. This chapter then goes on to define the role that a tumour growth model would play as part of an overall radiotherapy process model.

1.1 Focus Of This Work

This thesis describes the theoretical basis, development and testing of a **Double kinetic Gompertzian-Exponential (DGE)** tumour growth model for clinical research applications. This unique tumour growth model is designed to be both a spatial and a temporal model able to simulate tumour growth from the time of diagnosis to the beginning of cancer treatment as well as subsequent tumour growth during treatment.

The model is intended to provide a link between clinical spatial information and a radiobiological cell kill model which is being used as the basis of the overall radiotherapy process model being developed. In this thesis, I will present the mathematical development of the DGE model, investigate the behaviour of the model under various conditions and provide test results of the model by way of comparison with existing clinical and experimental data.

1.2 Project Rationale Of Overall Radiotherapy Process Model

The treatment of cancer by radiation therapy is a complex process. It is the assimilation and application of engineering, radiation physics, molecular biology, imaging, clinical judgement and radiation delivery technology. In the past three decades, many investigators have considered various components of the radiation therapy process including:

- The uncertainty of tumour target localization ^[1, 2];
- The influence of treatment planning and dose calculation techniques on dose distribution accuracy ^[3];
- The effect of patient immobilization devices on patient spatial dose distribution ^[4-7];
- Errors in the spatial accuracy of dose delivery equipment ^[8-12];
- Biological models of tumour and normal tissue response ^[4, 13-18].

These studies, aimed at the improvement in radiotherapy, have almost exclusively focused on the improvement of dose delivery. To date very little work has been done on assessing how potential changes in the overall process, for example physician training, methods of imaging or multidisciplinary clinics could effect a change in outcome, although Jones ^[19] suggested that significant improvements in cancer treatment could be gained by modeling the radiotherapy process as part of the design of a clinical trial. To date, most of the relevant information on tumour biology, accuracy, motion etc. stands in isolation and has never been coalesced into one model that could predict the effect of new technologies and treatment approaches on patient outcome. The purpose of our overall project is to develop a process model of radiation therapy, including physician training

and practice, patient setup, dose delivery, organ motion, imaging information and combine it with radiobiological response models and a general tumour growth model to predict population outcomes in terms of Normal Tissue Complication Probability (NTCP) and Tumour Control Probability (TCP). It is hoped that this could then provide a tool to help governments and industry optimize resource utilization in deciding on personnel training, purchasing new technologies and the focusing of research initiatives. A critical component of this overall radiotherapy process model is a temporal spatial tumour growth model which can be applied to predict the tumour change prior to and during radiation treatment. This tumour growth model is the focus of this thesis.

1.3 Historical Background of Radiotherapy

1.3.1 What Is Radiotherapy?

Radiotherapy, also called radiation therapy, is the treatment of cancer and other diseases with ionizing radiation. In this treatment, ionizing radiation deposits energy at the molecular level that destroys cells in the area being treated ("target volume ") ^[20]. Most lethal damage is thought to be focused on the genetic material, making it impossible for these cells to continue to reproduce (biological cell death) ^[21]. This damage occurs to both cancer cells and normal cells and clinically physicians often think in terms of TCP and NTCP to estimate damage to both normal tissues and tumour targets.

Currently, radiotherapy is used to treat localized tumours, such as cancers of the skin, tongue, larynx, brain, breast, or uterine cervix, as well as disseminated tumours such as leukemia and lymphoma (cancers of the blood-forming cells and lymphatic system, respectively) ^[22]. Target volumes (size of treatment fields) vary from very small stereotactic fields (1 cubic centimeter) to the Total Body Irradiation (TBI). Commonly radiation modalities include external beam, both electrons and photons, generated by mega-voltage and ortho-voltage electron accelerators, as well as internal or brachytherapy radiation from small encapsulated radioisotope sources as well as some unsealed systemic brachytherapy treatments.

1.3.2 History of Radiotherapy

Within a few months of Roentgen's discovery of X rays in 1895, their potential application for the treatment of disease was realized and tested by physicians of the time. The first documented x-ray treatment was performed and reported by Voight ^[23], a German physician, who treated a patient diagnosed with nasopharyngeal cancer on 3 February 1896. A lack of knowledge concerning dose and normal tissue response resulted in an unfavorable outcome for this treatment. The first successful treatment of cancer by radiotherapy was reported in 1899 ^[23], when two patients were treated in Sweden. The first was a patient treated for a squamous cell cancer on the cheek using a course of over 50 fractions, although subsequent surgery of a small resistant portion of the tumour was needed before cure could be claimed. The second was a basal cell cancer of the nose treated entirely by x rays. This patient, a 49-year-old woman, remained disease free for 30 years ^[24].

Since then, the use of radiation to treat cancer has grown steadily worldwide. At the same time the science of radiation therapy has evolved dramatically, including the development of exposure and absorbed dose units (International Commission on Radiological Protection (ICRP) and International Commission on Radiation Units and Measurements (ICRU)). A series of technical standards (task group series, AAPM and IAEA) have been published to guide cancer treatment by external radiotherapy. To date, about half of all cancer patients are candidates for radiotherapy to treat their disease ^[25].

1.3.3 The Evolution of Radiotherapy

Many important technological changes have been made and numerous developments have been accomplished since the first application of radiation to cancer treatment:

- The development of high energy radiation beams for more effective cancer treatment of deep seated tumours^[26];

- The development of versatile linear accelerators and patient couch designs so that radiation can be delivered from different angles and directions in three dimensional space^[26];
- The introduction of multi-leaf collimators (MLC) and other beam shaping devices for precision control and shaping of the radiation beam^[18];
- The development of patient immobilization and positioning devices to reduce patient, organ and tumour motion during treatment^[5];
- The use of CT, PET, MR and other image data systems to provide three-dimensional planning information to guide radiation treatment^[26, 27];
- Computer systems and networks used to plan and track radiation treatment, provide dose calculations and provide data links among the processes of treatment have been implemented in order to improve the efficiency and reduce human errors^[28, 29].

These changes and developments have all had the same goal — to effectively destroy the cancer tissue while delivering as little radiation as possible to the adjacent normal tissues.

1.3.4 Cost Escalation In Radiation Therapy

Health care systems worldwide face a huge challenge: the conflict between limited medical resources and a growing demand for medical care. Most governments are concerned with the increase in health care costs year by year^[30, 31]. A recent estimate shows that the annual cost for cancer treatment in the US is about 107×10^9 dollars and increasing by approximately 15% per year^[32, 33]. Investigation has suggested that the cost escalation in cancer treatment results from the introduction of new advanced equipment and the ever increasing wages of highly-qualified personnel as well as an increased incidence of disease. During the past three decades, a variety of new technologies have been introduced into radiotherapy such as advanced linear accelerators (linacs) and planning systems. These technologies are very expensive. For example, a modern linac capable of delivering IMRT (Intensity Modulated Radiation Therapy)

treatments costs in excess of \$2M. In addition, modern more sophisticated equipment requires highly qualified personnel to operate and is expensive to maintain.

1.3.5 Outcome

The combination of all of the new and advanced technologies mentioned above (imaging modalities, treatment planning computers, linacs) can significantly reduce the error in delivered radiation dose to the tumour targets. It has been a widely held belief that an improvement in the radiation delivery will translate into an increase in patient survival ^[34, 35]. Unfortunately, this expectation has not been realized for most types of cancer. Figure 1.1 shows the crude incidence rate and mortality rate in Canada from 1971 to 2000 ^[36]. The dotted curve and the solid curve in the figure are the number of new cases of cancer and cancer death respectively, expressed as a rate per 100,000. This

Figure 1.1 Incidence rate and mortality rate of cancer in Canada ^[36].

data shows that the overall ratio of survival to incidence has improved by only about 10% during the last 30 years. A recent investigation ^[37] has shown that the 5 year survival rate (Figure 1.2) for all cancer patients in the United States has improved from 49% in the early 1970s to nearly 60% currently. On the surface this may appear to be a significant

Figure 1.2 The five year survival rate change in USA ^[37]

change but it must be remembered that this is due to many factors including the improvements in early diagnosis and major changes in chemotherapy and surgery in the last 30 years and much of the overall change in survival can be attributed to these. It is very difficult to determine how much of the change can be attributed to radiotherapy alone, but it is unlikely that radiotherapy is the most significant among these factors.

1.4 The Overall Radiotherapy Process Model

The apparent lack of improvement in survival as a function of improved accuracy in dose delivery suggests that the development directions of the radiation therapy community may need to be refocused. The disappointing improvement in survival rate over the past three decades despite significant improvements in the dose delivery suggests that the entire radiotherapy process must be scrutinized, not just the dose delivery which has been the focus of medical physicists for a long time.

In order to examine the whole treatment process, it is important to consider the entire spectrum of variability, uncertainty and error inherent in the system. To date most research has focused on predicting the impact of error and variability on specific endpoints, such as dose distribution relative to bony landmarks^[38] but very little has been done in predicting how well the advance in dose delivery improves the outcome in terms of survival. No model has been developed which coalesces all of the variability, error and uncertainty in radiotherapy to provide a prediction of the impact of a process change on population outcome.

Such a model is currently under development in the medical physics Lab at the Ottawa regional cancer centre. The scope of the model covers the entire radiotherapy process from the initial patient presentation to the completion of radiation treatment. All sub-processes in radiotherapy treatment are connected to each other. In general radiotherapy is a complicated non-linear dynamic system. As a first attempt the model being developed will treat the process as a series of linearly related events. This has some merit in that other workers have treated parts of the process such as fractionation of dose delivery as a linear system which can be modeled by linear theory^[5, 39].

The radiotherapy process can be considered as such a system, where the input is a patient with a tumour cell distribution within their body and the output of the process is a patient with or without a sterilized tumour. It is an inherent belief of the system that patient outcome is determined at least in part by the treatment process, and hence it is the premise of this work that changes in the process must necessarily result in changes in

patient outcome. It is the goal of the global work to examine the whole process and determine the impact various components of the process have on patient outcome.

The radiotherapy treatment process is complex. The entire model must address temporal, spatial, radiobiological and process issues. In addition, this model should be capable of providing tools to assess the noise and the error generated and transmitted in each step of the process and the relative impact of this error on patient outcome.

Figure 1.3 is a flowchart illustrating the radiotherapy treatment process. Initially the patient presents clinically at time $t = 0$ with some absolute tumour cell density distribution $\rho(x, y, z, t_0)$, although it is not technically possible to exactly know what that tumour cell density distribution is. The initial part of the radiotherapy process consists almost entirely of information gathering, processing and transmission. This process includes such specifics as biopsy, pathology, imaging, image analysis, surgical report analysis, physical examination etc. The physician (radiation oncologist) assimilates this

Figure 1.2 Flowchart of the conceptual model of the radiotherapy process.

information and estimates a tumour cell density (TCD) distribution which he/she correlates to the patient anatomy with imaging studies such as CT and MRI. Up to this point the physician has been working in “Tumour Cell Density (TCD) Space”. The physician then mentally applies a transform function, which is patient and pathology specific to determine a desired dose distribution. Subsequent to this the radiotherapy process functions in “Dose Distribution Space”. Both the TCD and dose distribution have temporal and spatial components and the dose delivered may not act upon what is believed to be the tumour cell distribution.

The entire radiotherapy process model that we are developing describes how the whole radiotherapy process works, describes the tumour growth with time, and the tumour cell density information transmitted from one component to another. It also describes the dose distribution acting upon the distribution of the tumour cell density temporally and spatially.

The entire radiotherapy process model consists of three components. The first part describes the tumour growth during the radiotherapy process, the second part describes the radiobiological response of normal and tumour tissue and the third part focuses on the simulation of the radiotherapy process. It is the tumour growth model which is the focus of this thesis.

1.5 Role Of The Tumour Growth Model

The goal of radiotherapy is to destroy the tumour and all of the dose delivery related processes are based on an assumed temporally invariant spatial distribution of this target. Unfortunately the TCD is not temporally invariant. From initial presentation until the end of treatment the TCD changes. This tempo-spatial change in tumour target should have an effect on patient outcome. Since the purpose of the radiotherapy process model is to predict the impact of process change on outcome, it is necessary to include the effect of tumour growth during the treatment process.

Therefore, one of the keys to the success of this radiotherapy process model is to build a three dimensional, spatially variant, time dependent model of tumour growth that can be used to model changes in TCD during the entire radiotherapy.

1.6 Requirements Of The Tumour Growth Model

The aim of radiotherapy is to decrease the number of viable tumour cells to as few as possible. The number of tumour cells which remain after therapy is dependent upon how many cells there were prior to therapy. Thus, tumour growth prior to and during radiotherapy treatment is one of the critical factors which seriously impact patient outcome ^[40]. The overall radiotherapy process model requires a model to simulate tumour growth and this model must be a tempo-spatial model as discussed below.

1.6.1 The Model Must Be Spatial

In radiotherapy, when dose is delivered to the tumour, usually a dose distribution is prescribed for the tumour target and the prescribed dose distribution often depends upon the tumour cell distribution within the tumour. The higher the cell density in the tumour, the higher the dose required to sterilize it ^[5]. In most cases the estimated TCD is tagged to a three dimensional image (e.g. CT or MRI) of the patient. Further, the desired spatial dose distribution is defined from the same images. In the near future, functional imaging systems will display the pH, blood flow, oxygen and nutrient information on 3D images ^[26, 41].

To tie these things together, the radiobiological model must introduce spatially variant parameters and hence the tumour growth model which feeds it must be spatially variant. To apply this model to the radiation therapy process, it is necessary that the model be spatially oriented to the voxel size should correlate to that of the modalities used to initially define targets.

1.6.2 The Model Must Be Temporal

It is well known that there exists a waiting time from initial diagnosis to the time of dose delivery. This delay is necessary due to the complexity of the process but can be exacerbated due to the shortage of radiotherapy resources ^[40]. The total delay from

diagnosis to the beginning of treatment can be up to 2 months in some cases. In addition the treatment is fractionated and can be prolonged over a two-month period. During this time the tumour may grow significantly depending on the type and stage of the tumour. It has been suggested that excessive delays to the start of radiotherapy could result in loss of local tumour control ^[25].

Fractionation is commonly used in radiotherapy to spare normal tissues while still achieving a satisfactory tumour kill rate. However, fractionation introduces many problems in tumour kill modeling because the tumour size and state is not the same for each fraction, due to repair, reoxygenation, redistribution and regrowth of the tumour ^[25]. All these factors may change radiosensitivity. A viable radiobiological model must then allow for temporal changes in tumour sensitivity and hence the model of tumour cell distribution must be temporal.

In summary, the dynamic change of tumour cells in size or in number and dose fractionation during the treatment process requires that the tumour growth model must be a temporal model that can be applied to the overall radiotherapy process model.

1.6.3 The Model Must Behave As Seen Clinically

The tumour growth model should be able to mimic tumour growth that is observed clinically and in the laboratory. It must be able to generate symmetric spherical growth as is observed *in vitro* (e.g. spheroids) and in some solid tumours in animal models as well as humans. It must be able to imitate asymmetric and infiltrative growth seen clinically and must simulate non-contiguous primary tumour spread resulting in local regional nodal involvement.

1.7 Tumour Growth Models

The biological mechanisms involved in tumour growth are extremely complex. Intensive experimental and clinical research ^[42, 43] has been done over the last 30 years, but the complete biophysical mechanisms of tumour evolution are still not clear. To date, no one has proposed a tumour kinetic model that has been widely accepted, probably

because the dynamic process of tumour growth involves such diverse disciplines as genetics, biology, molecular biology, statistics and mechanics and investigators in the different fields often focus on their own area and ignore the influence from other fields.

Nonetheless, many attempts have been made to model some of the fundamental processes of tumour growth and spread for different applications. A variety of mathematical models ^[43-46] have been developed to simulate tumour growth both temporally and spatial-temporally. Probability models apply the Monte Carlo approach following the “game of life” to simulate tumour growth in a two or three-dimensional matrix. Diffusion models have been used to model the spatial growth of tumours usually assuming that tumour growth is a wave propagation phenomenon ^[43, 47, 48] and is driven by a mechanical-chemical framework.

Many of these models have not been tested against experimental data, especially the tempo-spatial tumour growth model. A reason for the lack of the testing might reside in the relative scarcity of reliable and complete tumour growth data that is critical for the checking of the reliability models. However, much of the experimental data that exists has been modeled using purely time dependent growth laws based on either exponential or Gompertzian growth ^[49-51]. These models are discussed more fully in Chapter 2.

1.8 Double Gompertzian-Exponential (DGE) Tumour Growth Model

The DGE tumour growth model, which was suggested and developed by myself and will be described fully in Chapter 3, is derived from the commonly used Gompertzian-Exponential growth model ^[50]. Our modification to this model introduces a spatial character and then differentiates between local and systemic feedback inhibition during tumour development. Our DGE model assumes that local feedback is at the voxel level and is dependent upon local factors such as pH, nutrient supply etc. and in fact is treated as the condensation of all local biological factors that inhibit tumour growth. It further assumes that systemic inhibition is associated with the overall patient tumour burden. In our DGE model we introduced not only a spatial character, but allow this character to be spatially variant. We suggest that cells in one voxel can spread to

adjacent voxels with a certain probability. The spread is driven by the tumour cell concentration gradient.

This DGE model will be shown to simulate various tumour growth patterns including symmetrical tumour growth such as bulk growth, asymmetrical growth such as at tissue interfaces and discontinuous growth such as local regional nodal involvement.

1.9 Outline Of The Thesis

The first chapter introduces the conceptual model of the overall radiotherapy process model and the requirements of a tumour growth model which is a part of the overall project. Chapter 2 then describes the fundamental cell and tumour kinetics and a variety of tumour growth models. Chapter 3 introduces the mathematical development of the Double Gompertzian-Exponential tumour growth model and the software ROSS designed to implement this model. Chapter 4 presents software tests, model test and the comparison of this model to the measured data for multi-cellular spheroid growth *in vitro* and a virtual untreated GBM brain tumour. Chapter 5 discusses its application in clinical physics and the advantages and disadvantages of this model. Finally, Chapter 6 concludes this thesis by summarizing the main results and the basic features of the DGE model and suggests possible future work.

CHAPTER 2

TUMOUR GROWTH MODELS

2.0 Overview Of This Chapter

This chapter introduces the subject of tissue tumours and their classification, and the fundamental kinetics cell of tumour growth. It then goes on to describe various tumour growth models and their advantages and disadvantages.

2.1 Tumours And Their Classification

The word tumour comes from the Latin for “swelling”, but not all tissue swellings are tumours. Tumour has been defined by the pathologist Wallace Clark ^[25] such that a fully developed tumour is a population of abnormal cells characterized by temporally unrestricted growth and the ability to grow in at least three different tissue compartments: the original compartment, the connective tissue of the primary site and a distant site away from the primary site.

Tumours can be divided into two categories according to their threat to life as either malignant or benign. In general, malignant tumours differ from benign tumours in four biological properties: structure, rate of growth, invasive growth and disseminated growth by metastasis. Metastatic ability ^[52] is a unique feature of malignant tumours. If a cancer has metastasized, treatment must be extended beyond the local region of the

primary tumour, usually in the form of combined modality treatment including chemotherapy and radiation.

2.2 Tumour Cell Kinetics

The growth rate of an entire tumour depends on three parameters: the average cycle time of the proliferating cells in the population, the growth fraction (percentage of cells within the population undergoing cell division), and the rate of cell loss from the population due to cell death. Biologically, the behaviour of tumour cells is very similar to that of normal cells except that tumour cells are abnormally regulated.

As shown in Figure 2.1, the cell cycle for tumour and normal cells can be divided into four phases: two functional phases S and M, and two preparatory phases G_1 and G_2 . For each cycle, the RNA and proteins are synthesized at G_1 phase, the DNA is synthesized in S phase, and the remaining RNA and proteins are synthesized in G_2 phase. During M phase mitosis or cell division is performed. Once a cell completes its division, if conditions are conducive to subsequent cell division, cells pass through the restriction point R and quickly move into S phase and then step into another cycle or they may temporally transfer to the quiescence state G_0 .

The cell cycle time is defined as the average time for a cell to successfully complete a process of **mitosis** without considering rest time at G_0 phase and is given by:

$$T_d = T_{G_1} + T_S + T_{G_2} + T_M \quad (2.1)$$

where T_d is the average cell cycle time, and T_{G_1} , T_S , T_{G_2} , T_M are times for the cell in each phase G_1 , S, G_2 and M respectively. T_d can be measured by means of the thymidine labelling index (TLI) method or autoradiography^[53]. Experiments have shown that there is a wide range of cell cycle times from less than 10 hours to more than 40 hours in cultured mouse tumours and from 72 to 260 hours for human tumours and varies with cancer type^[25].

In order to maintain tissue homeostasis and to support normal development, each organ maintains strict controls over their cycle time, growth fraction and cell loss. The

homeostatic control mechanism (HCM) of the human body is responsible for maintaining a balanced number of cells by regulating the cell proliferation process. Under normal circumstances the proliferation process is carefully controlled to ensure that there are not too many or too few cells generated to affect the function of the organ. But tumour cells, which are free of the homeostatic control ^[53], continue to proliferate if the environmental conditions permit.

Figure 2.1 The cell cycle.

2.3 The Objective of Mathematical Models for Tumour Growth

A good mathematical tumour growth model should help physicians and scientists to understand complex biological mechanisms, aid them in evaluating the stage of tumour growth, develop optimum treatment plans ^[54], and potentially improve cancer therapy.

In order to study the dynamics of tumour growth in cancer therapy, it is often easier and cheaper to simulate tumour growth with mathematical modeling than to

perform laboratory measurement. Modeling can also be much faster than clinical trials which may take several years. It can also be a much more practical and ethical way to evaluate new ideas and clinical approaches. Finally and most critically, it is very difficult, sometimes impossible, to obtain the relevant data from clinical research. For example, it is extremely difficult to dynamically measure the number of tumour cells in real patients directly. It is also extremely difficult to obtain sufficient data on untreated tumour growth for radiotherapy research purposes.

Generally a tumour growth model describes the dynamic development of the cancer cell population. A model can be applied to estimate the optimal parameters of acceptable dose of radiotherapy. It may also help to determine the CTV (Clinical Tumour Volume) after determination of GTV (Gross Tumour Volume) from imaging studies and other investigations ^[20].

2.4 Overview Of Tumour Growth Models

General speaking, tumour growth models can be classified into five categories: probabilistic models, empirical models, structural models, diffusion-interaction models and fractal models.

2.4.1 Probabilistic Models

Mathematicians Von Neumann and Ulam attempted to develop the first probabilistic tumour growth model in the late 1950s ^[55]. These models rely on the randomness of many natural phenomena and assume that cell division is a Markov process. They consider cell locations as squares on a matrix and each location may be “empty” or may contain a cell. A cell whose surrounding area is empty may divide. Cells are chosen to divide by a probability based on cycle time. An algorithm is also created for the spread of tumour cells. As shown in Figure 2.2, the initial step of the probabilistic model is to choose a suitable size, say, a 100×100 two-dimensional array, which is initialized with all elements set to contain 0. A start cell is placed in the centre of the array, and marked as 1. The next step is to make growth rules to enable the single

start cell to divide and spread. Cells may spread to one of the four adjacent directions. When only one cell is present, there is only one choice as to which is the next cell to divide. Division takes place by making one of the neighbouring array sets equal in value to the dividing cell.

Figure 2.2 Biological “cell” as squares on a matrix. The initial cell is marked by the circle in the centre of the matrix, “0” means no cell occupation, “1” means cell occupation.

The original site becomes one of the two “daughter” cells, the two “daughter” cells will divide and search empty space following the same rules. These early probabilistic models can simulate some observed cell division patterns depending on the rules invoked. The various division and spread rules produce different results and may help to indicate the roles of various factors acting in a real system. Unfortunately,

these models have little biological meaning and can only simulate some of the general behaviour characteristics of tumour growth.

Cellular automaton (CA) models have been used to simulate tumour growth for a long time. It is assumed that tumours are discrete dynamic systems whose behaviour is completely specified in terms of a local relation. The implementation of cellular automaton to simulate tumour growth consists of a grid of cells that can change their state. Each time step a set of iteration rules is applied to each of the cells.

Based on automata theory, Duchting and Vogelsaenger developed a realistic spatial cell growth model^[54]. They assumed that the life span of a cell in the cell system is determined by a random number and a division of a cell takes place only when there is adjacent free space for one of the new daughter cells. If there are more daughter cells needing space, the mother cell is deleted in the cell space when its life span is over. The cell cycle intervals like G_0 , G_1 , M , G_2 , S are taken into account and the probability of cell loss is fixed at any time step. The duration of each individual cell phase is randomly assigned to each tumour cell following a Gaussian distribution. A cell can move from each cell phase to cell death directly. Although the model can predict useful information such as spatial sensitivity for chemotherapy and radiotherapy, the disadvantage is that the search of empty space takes a very long time, even for a small tumour with 10^7 cells (0.01 cm^3 in volume) and requires large computer memory.

A. R. Kansal^[45] introduced a Voronoi tessellation polygrid and Gompertzian growth laws into a three-dimensional cellular automaton to simulate tumour growth. This CA model can simulate three-dimensional tumour growth over any time using several microscopic parameters including cell doubling time, average overall tumour radius and proliferative rim thickness. It can simulate the tumour growth starting from a group of 1000 cells to the final stage of brain tumour with 10^{11} cells. It can also predict necrotic cells, non-proliferating cells and viable cells in a symmetric spherical tumour. Unfortunately this model can't simulate asymmetric growth such as that observed clinically at tissue interfaces nor does it simulate local regional nodal involvement.

A probabilistic model represents the tumour growth based on the stochastic behaviour of individual members of the cell population. It assumes that cell proliferation is a random and disorganized process. But most of the probabilistic models ignore the

systemic behaviour of tumour growth, which is determined by the patient burden. Thus, probabilistic tumour growth models are not considered for use in the overall process model.

2.4.2 Empirical Models

Empirical models, also called deterministic models, describe tumour growth based on the behaviour of large groups or classes of cell populations over long time periods. For bulk tumours, empirical models are based on clinical observations of the dynamics of tumours which are large enough to be directly measurable. These are only viable prior to therapeutic intervention and only when the tumours reach a size of roughly 1 mm or more in diameter.

Most clinical observations have shown that net tumour growth rate is a function of tumour volume. Usually empirical models apply first-order differential equations based on measurable quantities such as tumour volume or cell counts. Due to their relatively simple form, empirical models are often applied in the numerical solution of problems related to radiation therapy and chemotherapy (exponential ^[25] or Gompertzian growth ^[49, 50]). Generally speaking, empirical tumour growth models using first order Ordinary Differential Equations (ODE), oversimplify the dynamic system of tumour growth compared to other kinds of models. Moreover, they overemphasize the systematic behaviour of the tumour growth and generally ignore the local individual behaviour and heterogeneity of cells within a tumour. However, these models are widely applied and partially accepted by experimental biologists and clinicians. They are the most applied biological models in practice especially in the modeling of bulk tumour growth.

An empirical tumour growth model is useful to describe a relatively simple system but in most cases, tumour growth is a very complicated system. The clinical observation of tumour growth usually starts from the detectable lesion. Modeling such comparatively late stages is important in cancer treatment, but usually does not describe the tumour growth well at very early stages.

Empirical models to date have only provided dynamic growth without any spatial character, and therefore cannot simulate cell-cycle kinetic effects, multi-compartment systems, stochastic fluctuations in cell number, spatial heterogeneity and other details within the tumour. As such these models cannot be applied directly and hence the existing forms are not acceptable for use in the overall process model.

2.4.3 Structural Models

Structural models assume that the tumour is a perfect sphere and is composed of a necrotic core surrounded by a viable rim of proliferating cells. They assume that processes such as proliferation, necrosis, inhibition etc. are symmetric, and that tumour growth follows spherical symmetry laws at all times. Under these conditions the tumour's growth can be described by its radius if one knows the relationship among the radius of necrosis and tumour etc. Landry and coworkers^[56] developed a structural model for the growth of a multi-cellular spheroid. They assumed that the cycling cells are confined to the periphery of a shell defined by the maximum thickness over which cells can receive, by diffusion, sufficient nutrients to sustain normal proliferation during their development. The cycling cells in the viable rim grow exponentially and these cycling cells continue to be transferred from the outer rim toward the centre which is a net loss of cycling cells in the viable rim. They found a relationship between the growth kinetics of a larger spheroid and the thickness of the dividing shell, such that the diameter D of the spheroid is given as:

$$D = D_0 + cT \quad (2.2)$$

where D_0 is the initial diameter, T is the time of growth and c is a constant equal to the slope of the diameter growth curve. This model predicts an infinite expansion of the spheroid diameter and it does not explain the saturation of growth with the increase in spheroid volume.

The disadvantage of these models is that they are too artificial to define the relation among the radius of proliferation, necrosis and cell number. As such these structural models are not an appropriate choice for our entire process model.

2.4.4 Diffusion-Interaction Models

Diffusion-interaction models assume that there exist more than one clonal population within the tumour tissue, and each might have different potential doubling times, cell loss rates and nutritional needs. These models simulate the competition of normal cells and tumour cells which is described by the classical Lokta-Volterra equations ^[45] given as Eq.2.3

$$\begin{cases} \frac{\partial N_1}{\partial t} = r_1 N_1 \left(1 - \frac{N_1}{K_1} - \alpha_{12} \frac{N_2}{K_2}\right) \\ \frac{\partial N_2}{\partial t} = r_2 N_2 \left(1 - \frac{N_2}{K_2} - \alpha_{21} \frac{N_1}{K_1}\right) \end{cases} \quad (2-3)$$

in which N_1 and N_2 are two different populations (e. g. tumour cells and normal cells), and the parameters r_1 and r_2 are the intrinsic rates of growth of each population. The parameters K_1 and K_2 are the maximum number of individuals in each population which could occupy a given volume and be sufficiently supported by the environment in the absence of the competing populations. The parameters α_{12} and α_{21} are the competition coefficients representing the effects on N_1 (or N_2) caused by the presence of N_2 (or N_1).

In their simulation, two cell populations share the same resources and compete with each other. Gatenby ^[57] presented a tumour – host Lokta-Volterra interaction model in which he added a diffusion term into the Lokta-Volterra equation and assumed that the diffusion of tumour cells is affected by the concentration of glucose ^[58]. His modifications of the Lokta-Volterra equation are based on the fact that some tumour cells can survive when the pH is below 6.8 and that normal cells cannot survive in the interface area between normal tissue and tumour tissue. Although this model describes

the interface between host and tumour very well, it can't be used to simulate the entire tumour development.

Burgess and coworkers ^[59] suggested that the process of tumour growth is the interaction process between growth rate and the diffusion of tumour cells. Their equation expressing tumour growth is given as:

$$\frac{\partial n(r,t)}{\partial t} = D_c \nabla^2 n(r,t) + \rho n(r,t) \quad (2.4)$$

where $n(r,t)$ is the cell density at time t and radius r . The parameter ρ represents the net growth rate of cells and D_c is the diffusion coefficient. They assumed that tumour growth is spherically symmetric and the growth medium is homogeneous. Applying unbounded conditions, the solution to Eq.2.4 is given by:

$$n(r,t) = \frac{N_0 e^{\rho t} e^{-r^2/4D_c t}}{8(\pi D_c t)^{3/2}} \quad (2.5)$$

where N_0 is the initial number of tumour cells.

Some diffusion models assume that tumour growth is dependent upon vascular development. Maggelakis and other investigators developed a model using the production of tumour angiogenesis factor (TAF) by using a diffusion equation in a spherical geometry. This model can be used to simulate how the growth factor and angiogenic factor affect tumour growth. The description of these models can be found in reference 43,47 and 48.

Clinical radiotherapy application of these models has been limited by the complexity of cell interaction and growth kinetics.

2.4.5 Fractal Models

Fractal models assume that tumour growth follows a fractal pattern ^[46], based on the observation that most cancers are composed of repeating elements (such as tree patterns or multiple nodules) which are self-similar over various phases and sizes ^[25].

The dimension of a fractal is called its mass dimension. A mass dimension of 3 means that tissue structure is solid, and a mass dimension of 2 means that cells spread in a plane. The average mean mass dimension for a normal tissue is about 2.7. As a fractal geometric pattern, the number of cells is proportional to the tumour volume raised to a power ≤ 1 , that power being a function of the mass dimension. A small mass dimension produces a low power constant and a low ratio of the number of cells per volume of tumour. Simon Cross ^[60] suggested that the fractal concept could be usefully incorporated into models of biological process including cell growth and blood vessel growth. Onna Craciunescu ^[61] suggested that there might exist a correlation between the fractal characteristics and tissue type, which has the potential to discriminate between normal and tumour tissue. The challenge of fractal models lies in producing closer linkage between these models and the biology.

CHAPTER 3

THEORETICAL DEVELOPMENT OF A DOUBLE GOMPERTZIAN EXPONENTIAL GROWTH MODEL

3.0 Overview of This Chapter

Many tumour growth models were introduced in Chapter 2. Of all the tumour growth models, only the empirical models are widely employed in cancer therapy. Unfortunately, these models are only temporal, which cannot satisfy the requirements of radiotherapy process simulation for spatial character. The Double Gompertzian Exponential (DGE) model, a tempo-spatial voxel based model that is extended from the single kinetic Gompertzian-exponential model but with spatial character and the ability to simulate various tumour spread patterns, is described in this chapter.

3.1 Tumour Spread Patterns

The spread of tumour cells is an extremely complicated process and a detailed description of the spread mechanism of tumour cells is beyond the scope of this thesis. Basically we can categorize the spread into three categories: local spread (or bulk growth), regional discrete non-contiguous spread and distant spread (metastasis).

Figure 3.1 illustrates the simplified spread processes of tumour cells within the human body. For local spread, the spread of tumour cells is constrained in a stroma (or capsule). Regional spread occurs when some of the tumour cells break out the barrier of

stroma and successfully perform regional seeding. A few tumour cells can migrate from the primary (local) tumour into the circulatory system and seed in a distant organ, which is referred to as metastasis.

Since radiotherapy is usually used to treat bulk tumours, the tumour growth model developed here mainly focuses on the local tumour spread, although the model is capable of predicting the occurrence probability of regional and distant spread.

This model addresses four types of tumour growth/spread patterns, which have been observed clinically. These are bulk/symmetric tumour growth, asymmetric growth, local regional nodal involvement, and distant seeding such as metastasis.

3.1.1 Bulk/Symmetric Tumour Growth

In bulk/symmetric growth, tumour cells are considered to originate from a single ancestor tumour cell, then divide and spread homogeneously within the patient tissues. In this particular case the spread and growth are constrained within a stroma. No tumour cells are allowed to spread out of the stroma. Since there is only one cell type in the bulk, the potential doubling time for the tumour cells is a constant.

The Double GompEx model, which will be fully introduced in Section 3.4, is applied to simulate the bulk tumour growth. It is used to address the non-homogeneous nutrient supply and cell density inside a voxel and heterogeneous character of the whole tumour during the tumour development. We assume that the natural behaviour of the bulk tumour is to grow symmetrically and that in the absence of external forces the shape of the tumour is a perfect sphere.

3.1.2 Asymmetric Growth

A basic character of human anatomy is the separation of structures and the variation in the properties of adjacent structures. Tumours originate in a single compartment or stroma. When a tumour grows it will encounter these boundaries and it has been observed that they preferential spread along or into some structures over others. For example when the periphery of a tumour is close to a blood vessel or the lymphatic

system, the tumour will prefer to spread along them. On the other hand, when the periphery of some tumours meets bone, spread in that direction is retarded. A specific example of this preferentially spread can be observed in prostate cancer, where the disease is more likely to spread along the seminal vesicles.

In the DGE model being described here, the individual structures are predefined and are provided with individual characteristics. These characteristics include the probability of spread. In a bulk symmetric tumour this probability would be spatially invariant, but with asymmetric spread the probability varies from one structure to the next.

3.1.3 Local Regional Nodal Involvement

Local regional nodal involvement is observed clinically for most tumours. There are two known pathways for this type of seeding to occur. During tumour growth, some tumour cells leave the primary tumour and are transported a short distance away by the lymphatic system. They are then trapped in local lymphatic nodes and start to grow and spread under appropriate conditions. The little spots shown in Figure 3.1 (c) represent these local regional seeds. Another pathway of local spread has been attributed to surgery. During the surgery, some tumour cells are dislodged or attach to the surgical instruments and are deposited in local surrounding tissues. After surgery these tumour cells could survive and lead to the local regional seeding ^[25].

It is difficult to detect small local seeding, although advances in nuclear medicine are making this possible for some disease sites such as breast cancer. These seeds have the potential to be a source of local treatment failure, and as such are critical targets in radiotherapy.

In our DGE model, voxels representing lymphatic nodes are selected and a small but finite probability of seeding is assigned to them. In each time increment of the calculation, seeding is determined by random number generation and is a function of the number of new cells produced in the bulk tumour during each time interval.

(a). Bulk/symmetrical growth

(b). Asymmetrical growth

(c). Local regional seeding

(d). Metastasis

Figure 3.1 Illustration of tumour spread patterns

3.1.4 Metastasis

The metastatic process is a complicated process, which occurs not only locally but also globally. As shown in Figure 3.1 (d), once some tumour cells break out of the primary tumour, they can enter the vascular or lymphatic circulation. Eventually they come to rest in a distant vascular or lymphatic bed, at which point they can actively extravasate into the organ parenchyma and proliferate as a secondary colony ^[25]. Metastasis is one of the most life-threatening aspects of cancer. Once metastasis occurs, it is impossible to cure the patient by local therapy alone. Our model is only concerned about whether or not a metastasis occurs, but once it occurs the growth of the metastasis is not considered in our DGE model.

3.2 Assumptions of the DGE Model

When simulating a complex biological or physical phenomenon, it is usually necessary to simplify the system in order to describe it by a mathematical model. In the DGE model, the following assumptions are made:

- The patient and tumour are regarded as a rigid body. There are no body changes and no deformation during the simulation period, so that the tumour can be described by a three dimensional matrix;
- The tumour is only composed of one type of cell with the same behaviour, so that one potential doubling time can be applied to all tumour cells. The potential doubling time T_{pot} is the shortest possible doubling time for the cell type, that is, the doubling time under perfect conditions;
- Tumour cells maintain a dynamic equilibrium over a short time interval. At one time they may exchange with tumour cells from adjacent voxels. For a specific voxel, the number of tumour cells that migrate in is equal to the number of tumour cells that migrate out.

3.3 Cell Growth Curves

A mathematical function called the growth curve describes the increment in the number of tumour cells, or the size of tumour as a function of time. This curve can provide dynamic information on tumour growth, and is very useful in helping plan a strategy for cancer treatment. Historically, many models have been used to describe this growth. Among them, exponential and Gompertzian growth curves have been widely used by medical physicists and radiation oncologists to describe tumour growth in size and volume.

3.3.1 Exponential Growth

When modeling the growth of a population of cells, it is common to assume that every individual cell doubles with every cell cycle. For example, the daughter cells themselves divide upon completion of the next cell cycle. This is typically expressed as an exponentially growing population. The use of the exponential growth model was first suggested by investigators at the South Research Institute in UK^[53]. It was based on the observation that leukemia L1210 grows exponentially until it reaches a lethal volume of 10^9 cells. The exponential growth model can be described by the following equation:

$$N = N_0 e^{\frac{t}{T_d}} \quad (3.1)$$

where N_0 is the number of initial tumour cells and T_d is the doubling time. The doubling time is the time for the tumour to double its number of cells. In this simple model, T_d is the only parameter used to describe the exponential growth model.

In this model, it is assumed that a cell has a constant doubling time, so that the tumour has a constant growth fraction without tumour cell loss. For example, if it takes 48 hours for 100 cells to grow into 200 cells, it will take 48 hours for 1000 cells to grow into 2000 cells.

3.3.2 Gompertzian Growth

The exponential model is simple and useful, but does not accurately described clinically observed tumour growth. For example, the growth fraction changes with time for almost all tumours and tumour cell loss does exist. Gompertz first introduced a modified exponential growth pattern in 1825 and now it is widely used in cancer therapy. With its feedback inhibition, the Gompertzian growth model describes the tumour growth better than exponential growth kinetics.

Figure 3.2 Tumour growth as a function of time.

In the Gompertzian growth model, the effective doubling time increases steadily as the tumour grows larger. There also exists a plateau to total growth given by $N_{\max}$. The tumour can asymptotically approach this size but never reach it. The general equation of the Gompertzian growth can be given as:

$$N = N_0 e^{\{k[1-\exp(-\frac{t}{T_d})]\}} , \quad (3.2)$$

where $k = \ln[N_{\max} / N_0]$, and T_d is the cycle time of the tumour cells. The variable N_0 is the number of initial tumour cells. As shown in Figure 3.2, the main feature of the Gompertzian growth model is its asymptotic behaviour, which implies that tumour size increases steadily but growth rate decreases as a function of tumour in size. In the Gompertzian model this is attributed to the fact that after the number of tumour cells reaches a certain value, the tumour growth fraction decreases, cell loss increases, and nutritional depletion occurs when the number of tumour cells outgrows the available blood supply.

The differential Gompertzian-exponential equation is given as:

$$\frac{dN}{dt} = (k_b - k_l)N , \quad (3.3)$$

where N is the number of tumour cells, k_b is tumour cell growth rate (hour^{-1}), k_l is the tumour cell loss rate (hour^{-1}). As shown in Eq.3.3, the tumour growth is determined by growth rate and loss rate.

For convenience, a biological concept, the potential doubling time (T_{pot}) of tumour is introduced into this tumour growth model. The potential doubling time T_{pot} is defined as the time required to double the number of clonogenic cells with no cell loss during the division. In this case the potential doubling time is equal to the average cycle time of tumour cells, under perfect growth conditions, which is the time for a tumour cell to successfully complete its division. The potential doubling time, T_{pot} , is a constant parameter in this model and is determined by the inherent nature of a specific line of tumour cells. The relation between T_{pot} and k_b is then defined by:

$$k_b = 0.693 / T_{\text{pot}} . \quad (3.4)$$

The average cell loss rate is defined by:

$$k_l = \begin{cases} 0 & N < N_g \\ \frac{\ln \frac{N}{N_g}}{\ln \frac{N_{\max}}{N_g}} & N > N_g \end{cases} \quad (3.5)$$

in which, N_g is the “Gompertzian Number” and is the critical number of tumour cells above which the tumour growth rate is feedback inhibited. Growth rate then asymptotically approaches zero as the number of tumour cells approaches some maximum value $N_{\max}$.

Since the single kinetics Gompertzian-exponential or GompEx model has been widely fitted to the existing data, a value for N_g , the global GompEx number, has been suggested by many investigators. Demicheli^[51] suggested that the transforming point from the exponential phase to the Gompertzian phase occurs when the number of tumour cells reaches 10^2 to 10^4 for spheroid growth. He also found that this value depends on cell type. The characteristics of Gompertzian phase are greatly dependent on the environmental conditions, such as the oxygen diffusion, pH, etc. Norton showed that the Gompertzian model can be made to fit some clinical data for the growth rate of breast tumours^[50].

All observed decrease in cell growth is handled as a cell loss k_l , which is the only feedback parameter in the GompEx model. It in fact represents more than cell loss and is the condensation of all factors which reduce overall growth rate such as a spatially variant reduced cycle time in the tumour. The Gompertzian growth model has been shown to accurately model the growth kinetics of some tumours^[49, 50]. However, it is only a temporal model, and the spatial characteristics of tumour growth are not described at all.

3.4 Double Gompertzian Exponential Model

In many circumstances, not all the cells are in the division cycle, but are in fact in the quiescent phase (G_0). The overall observed rate of population growth is affected by this and by the loss of cells. The cell loss and growth rates in a tumour are spatially variant ^[25]. This is extremely important to tumour dynamics, but is not described by the single GompEx model. In order to overcome this shortfall, we have developed a Double GompEx (DGE) tumour growth model.

This is a spatially variant voxel based model. It provides a mechanism for contiguous tumour spread from one voxel to any of its neighbour voxels as well as a mechanism for remote seeding of non-adjacent voxels. In the DGE model, we assume that both systemic factors and the local microenvironment affect tumour growth. The single kinetic GompEx growth is expanded into two nested GompEx growth functions, one reflecting the impact of the systemic effects which alter the growth of the entire tumour and the other reflecting local factors which affect only the growth rate in the local voxel.

Changes in growth rate, either locally or globally, are controlled by feedback kinetics. Feedback inhibition to growth at the local voxel level is considered as the condensation of all local biological factors which inhibit tumour growth such as interstitial pressure, lack of blood flow, the diffusion limit for oxygen, lowered pH, and cell loss due to apoptosis, exfoliation or immunologic attack. Global or systemic feedback is based on the concept of overall tumour burden in the host. It is assumed that individually, each of these obeys growth laws described by the single GompEx equation. The basis of these assumptions is described as following.

Tumour growth is observed to follow an exponential growth law during early stages under ideal conditions, i.e. sufficient nutrients for tumour growth, ideal pH, etc. However when the tumour radius exceeds 5~10 times of the size of a tumour cell the rate of growth is observed to decrease. This decreased growth rate has been attributed to many factors including an increase in interstitial pressure and the inability of sufficient nutrients to diffuse to the centre of tumour. In our DGE model we extend this global

observation to each single voxel inside the tumour, as is done in most Cellular automaton models. It is assumed that the conditions for tumour growth within a specific voxel are homogenous, but each voxel has its own growth kinetics governed by its own local environment. In addition growth in all voxels is affected by systemic retardation.

In the DGE model we model this by introducing two separate Gompertzian numbers, N_g and N_{gvoxel} . They represent the global and local trigger levels for feedback inhibition respectively. Similarly, there are global and local limits to the maximum number of tumour cells, N_{max} within the whole tumour and $N_{max voxel}$ within one voxel respectively. Thus, assuming that at some time t_0 we know the tumour cell density N_{i,j,k,t_0} in some voxel (i, j, k), the increase in the number of tumour cells in each voxel can be calculated using a numeric approximation to the DGE model given as:

$$\frac{\Delta N_{i,j,k,t}}{\Delta t} = \left[k_b - k_{l global} - k_{l(i,j,k)} \left(\frac{k_b - k_{l global}}{k_b} \right) \right] N_{i,j,k,t}$$

where :

$$k_{l global} = \begin{cases} 0 & \sum_{i,j,k} N_{i,j,k,t} < N_g \\ k_b \left(\ln \left(\frac{\sum_{i,j,k} N_{i,j,k,t}}{N_g} \right) / \ln \left(\frac{N_{max}}{N_g} \right) \right) & \sum_{i,j,k} N_{i,j,k,t} > N_g \end{cases}$$

$$k_{l(i,j,k)} = \begin{cases} 0 & N_{i,j,k,t} < N_{gvoxel} \\ k_b \left(\ln \left(\frac{N_{i,j,k,t}}{N_{gvoxel}} \right) / \ln \left(\frac{N_{max voxel}}{N_{gvoxel}} \right) \right) & N_{i,j,k,t} > N_{gvoxel} \end{cases}$$

(3.6)

where:

Δt is the time increment, and is small compared to the potential doubling time of the tumour.

N_{gvaxel} is the Gompertz number at the voxel or microscopic level. When the number of tumour cells in one voxel is greater than this value, local growth will transition to Gompertzian growth from exponential growth. Similarly N_g is the global Gompertz number such that when the total number of tumour cells summed over all voxels exceeds this number the growth of all cells will be feedback inhibited.

$N_{max\ voxel}$ is the maximum number of tumour cells allowed in each voxel. It can be determined by multiplying the voxel volume by the packing density of tumour cells (cells/mm³). Generally, 1 cm³ contains approximately 10⁹ tumour cells.

N_{max} is the maximum number of cells allowed in entire tumour.

In our DGE model, it is assumed that growth and spread of tumour cells are two independent processes and are calculated separately after each time increment Δt ($\Delta t \ll T_{pot}$). Growth in each voxel is as described in eq 3.6, while spread is driven by the concentration gradient of tumour cells between adjacent voxels.

After each time increment Δt , the increment of tumour cells in voxel (i,j,k) is given by:

$$\Delta N_{i,j,k,t} = \left[k_b - k_{l\ global} - k_{l(i,j,k)} \left(\frac{k_b - k_{l\ global}}{k_b} \right) \right] N_{i,j,k,t} \Delta t \quad (3.7)$$

where $N_{i,j,k,t}$ is the number of tumour cells in voxel (i,j,k) prior to growth.

In the DGE model we assume that when the number of tumour cells in one voxel exceeds N_{gvaxel} , some of the tumour cells may leave the current voxel and spread into an adjacent voxel. It is further assumed that only new tumour celis ($\Delta N_{i,j,k,t}$) are eligible to spread into the adjacent voxels. The probability of tumour cells leaving a specific voxel is defined by P_{spread} and is given as:

$$P_{spread} = \frac{k_{i,j,k}^{out} N_{i,j,k,t}}{N_{max\ voxel}} \quad (3.8)$$

where $k_{i,j,k}^{out}$ is a spatially variant constant that constrains the tumour growth ($0 < k_{i,j,k}^{out} < 1$).

This constant is spatially variant to reflect properties of different tissues and is equivalent to the diffusion coefficient in the diffusion tumour growth models.

The number of tumour cells which then spread to the adjacent voxels is determined by the number of new tumour cells born during time increment Δt and the spreading probability P_{spread} , and is given as:

$$\xi_{i,j,k} = P_{spread} \Delta N_{i,j,k,t} \quad (3.9)$$

where $\xi_{i,j,k}$ is the number of tumour cells that spread from the original voxel to the adjacent voxels after each time Δt .

In a homogenous medium, the spread of tumour cells is driven by the radial concentration gradient. Kansal et al have suggested that this is not a random, disorganized process, but that tumour cells form an “opportunistic” system^[45] and they are self organized. They search available space in which to spread, survive and grow. Furthermore, when they spread, they have “knowledge” of the environmental conditions in adjacent locations and they preferentially spread to new locations with relatively better environmental conditions than their original one. Within the DGE model itself, tumour cells are allowed to spread only to adjacent voxels. Distant spread is handled by a separate mechanism within the model.

All cells, $\xi_{i,j,k}$, which move out from the current voxel, must migrate to adjacent voxels. The probability that cells are assigned to a specific adjacent voxel depends on the capacity of that voxel to accept new cells. This is modulated by a randomized variable following a normal distribution.

Since it is very difficult to illustrate the spread of tumour cells in a three-dimensional matrix, the spread of tumour cells in a two dimension matrix is illustrated in Figure 3.3 instead. Referring to this figure, cells located in the centre of the matrix can spread to adjacent pixels. The relative probability of spread to a voxel on a principal axis (A1, A2, A3, A4) in the figure is 1.0, and to the diagonal directions (D1, D2, D3, D4) is 0.087. The derivation of these values is given later.

Figure 3.3 Illustration of tumour cells spread to 8 adjacent pixels in a two-dimensional plane.

The capacity $C_{i,j,k,t}$ of voxel (i,j,k) to accept tumour cells is given as:

$$C_{i,j,k,t} = N_{\max \text{ voxel}} - N_{i,j,k,t} \quad , \quad (3.10)$$

where, $N_{\max \text{ voxel}}$ and $N_{i,j,k,t}$ are the maximum number and current number of tumour cells in voxel (i,j,k) respectively. The total capacity for all adjacent voxels is:

$$\psi = \sum_{m=-1}^{m=1} \sum_{n=-1}^{n=1} \sum_{l=-1}^{l=1} v_{l,m,n} C_{i-m,j-n,k-l,t} , \quad (3.11)$$

where $v_{l,m,n}$ is a geometry factor defined by Eq.3.12, and is discussed in detail later in this chapter.

$$v_{l,m,n} = \begin{cases} 1 & |m| + |n| + |l| = 1 \\ 0.1418 & |m| + |n| + |l| = 2 \\ 0.0044 & |m| + |n| + |l| = 3 \end{cases} . \quad (3.12)$$

Of the tumour cells that leave the current voxel, the fraction of those cells that go to a specific adjacent voxel (i-m, j-n, k-l) is proportional to the capacity of that adjacent voxel to accept new tumour cells. This fraction is given by:

$$k_{i-l,j-m,k-n}^{in} = k_{i-l,j-m,k-n}^{spatial} \frac{v_{l,m,n} C_{i-l,j-m,k-n,t}}{\psi} , \quad (3.13)$$

where $k_{i-l,j-m,k-n}^{spatial}$ is a coefficient set to 1 to provide symmetric growth for the same tissues and structures and is spatially variant when asymmetric growth is simulated. It mainly depends on the voxel size, cell type, and local tissue architecture. A big value should be set to $k_{i-l,j-m,k-n}^{spatial}$ for the simulated voxel in tissue close to blood vessel and a small value should be set to it for the simulated voxels in bone.

The number of tumour cells that migrate to any adjacent voxel (i-m, j-n, k-l) from the current voxel is given by:

$$\phi_{i-l,j-m,k-n} = \xi_{i,j,k} k_{i-l,j-m,k-n}^{in} . \quad (3.14)$$

The number of tumour cells that are generated in the current voxel and do not leave it, can be described by the following equation:

$$\delta_{i,j,k} = \Delta N_{i,j,k,t} - \xi_{i,j,k} . \quad (3.15)$$

After each time increment Δt of tumour growth and tumour spread, the total number of tumour cells in one voxel is represented by the following equation:

$$N_{i,j,k,t+\Delta t} = N_{i,j,k,t} + \delta_{i,j,k} + \phi_{i,j,k} . \quad (3.16)$$

It is important to note that $\phi_{i,j,k}$ is the total number of cells which have migrated from the 26 adjacent voxels to voxel(i,j,k).

The Geometry Correction Factor $v_{l,m,n}$ introduced above needs special attention. The spread of a tumour is isotropic in an idealized homogenous environment, but the grid behaviour in a Cartesian coordinate system is not radially symmetric. To overcome this problem, the proportionate fraction of tumour cells which spread to different directions must be adjusted.

Because of the complicated three-dimensional geometry, it is difficult to calculate the spread weight numerically. In this work a Monte Carlo approach has been used to calculate this correction factor. In the Monte Carlo simulation, it was assumed that the tumour cells are distributed uniformly within a unit sphere. The unit sphere is centred at the coordinate (0, 0, 0) with a radius of 1 in a Cartesian system. In which there is also a unit voxel centred at the coordinate (0, 0, 0), and surrounded by 26 adjacent unit voxels. These 27 voxels overlap with the unit sphere. The overlapped volume between each of the 26 surrounding voxels and the unit sphere is considered as the spread weight for the tumour cells in each direction.

The unit sphere is described by the following equation:

$$r^2 = x^2 + y^2 + z^2 \leq 1 . \quad (3.17)$$

Since the cells are homogeneously distributed within the unit sphere, the density distribution function of tumour cells at r is

$$f(r) = 3r^2 \quad (0 \leq r \leq 1) , \quad (3.18)$$

and the density distribution of directional cosine

$$f_{\Omega}(\cos\theta, \varphi) = \frac{1}{4\pi} , \quad (3.19)$$

where $\cos\theta \in [-1,1]$ and $\varphi \in [0, 2\pi]$.

During the simulation, random points are sampled inside the unit sphere according to the distribution Eq.3.18 and Eq.3.19. The ratio of those points which fall inside each voxel to the total number of points is regarded as the overlap volume between each voxel and the unit sphere, i.e., the spread weight of tumour cells in each directions.

By applying the Monte Carlo sampling technique, r , θ , φ can be sampled according Eq.3.18 and Eq.3.19 :

$$r = \max(\xi_1, \xi_2, \xi_3) , \quad (3.20)$$

$$\cos\theta = 2\xi_4 - 1 , \quad (3.21)$$

$$\sin\varphi = \sin 2\pi\xi_5 , \quad (3.22)$$

$$\cos\varphi = \cos 2\pi\xi_5 , \quad (3.23)$$

where $\xi_1, \xi_2, \xi_3, \xi_4, \xi_5$ are random numbers between 0 and 1.

The coordinate of each point sampled in the spherical coordinate system is transformed to the Cartesian coordinate system using Eqs.3.24 to 3.26:

$$x = r \sin\theta \cos\varphi , \quad (3.24)$$

$$y = r \sin\theta \sin\varphi , \quad (3.25)$$

$$z = r \cos\theta . \quad (3.26)$$

The boundary of each voxel is defined as:

$$x_{ijk} - 0.5 < x < x_{ijk} + 0.5 , \quad (3.27)$$

$$y_{ijk} - 0.5 < y < y_{ijk} + 0.5 , \quad (3.28)$$

$$z_{ijk} - 0.5 < z < z_{ijk} + 0.5 , \quad (3.29)$$

where, $(x_{ijk}, y_{ijk}, z_{ijk})$ is the centre of each voxel:

$$x_{ijk} = i , \quad (3.30)$$

$$y_{ijk} = j , \quad (3.31)$$

$$z_{ijk} = k , \quad (3.32)$$

where $i, j, k \in [-1, 1]$. A point which falls into the central voxel (0,0,0) is not accepted, since the points inside 26 adjacent voxels are desired in this sampling.

Using the sampling technique mentioned above, if one point is sampled inside the unit sphere and it falls inside a specific voxel (i, j, k) , then the counter $\eta_{i,j,k}$ for this voxel would increase 1:

$$\eta_{i,j,k} = \eta_{i,j,k} + 1, \quad \forall (i, j, k) \neq (0,0,0) . \quad (3.33)$$

There were 10^6 such points sampled in the simulation. The absolute weight of each voxel is calculated as:

$$\tau_{i,j,k} = \frac{\eta_{i,j,k}}{10^6} . \quad (3.34)$$

and the statistics error for $\tau_{i,j,k}$ is estimated by:

$$\sigma_{\tau_{i,j,k}} = 0.001 \sqrt{\frac{10^6 - \eta_{i,j,k}}{\eta_{i,j,k}}} . \quad (3.35)$$

It was found the statistics error of weight was $< 1\%$. The 26 adjacent voxels are divided into three groups including axes, two-dimensional diagonal directions and three-

dimensional diagonal direction. The $\tau_{i,j,k}$ were renormalized to the weight in the principal axes directions, and the geometry correction factor for each voxel is calculated to be 0.0044 for three-dimensional diagonal spread, 0.1418 for two-dimensional diagonal spread, and 1 for principal axes spread.

3.5 Software Description

A computer program to execute the tempo-spatial DGE tumour growth model was developed under the Windows™ platform using Microsoft Visual Basic (version 6.0)™, Visual C++ (version 6.0)™ and Matrix for VB (compatible with MatLab™). This program was run on a pentium II with a 200Mhz CPU and a 64M RAM, under this condition, it took two hours to simulate a GBM brain tumour in its full history with a 2 mm voxel size, it will be discussed in Section 4.3.4.

The resulting software package, called ROSS (Radiation Oncology Simulation System), consists of three modules: the input-output module ROSS_IO, the calculation core module 3D_growth, and the Graphic display module Growth_Display. The structure of the software is shown in Figure 3.4.

Figure 3.4 Functional block diagram of the software

The module ROSS_IO, developed with VB, is the user interface of the software and is shown in Figure 3.5. It provides a user friendly window to provide input of the fundamental parameters and to display the results of the modelling calculation. This

module provides the opportunity for the user to load an initial tumour cell density distribution to act as the starting point of calculation and to save the results of calculation at any specified time.

Figure 3.5 A screen capture of the ROSS_IO interface for the DGE model

Figure 3.6 is a flowchart of the module ROSS_IO. First, it requires the user to load a three-dimensional tumour density file, and related tumour growth information such as voxel size, time for tumour to grow, potential doubling time of tumour cells etc. It then requires the user to enter other parameters such as growth time etc. ROSS_IO then checks to ensure that all the inputs are reasonable. If there exist conflicts among the parameters, the user is notified to modify the parameters until the software accepts them.

The 3D_growth module is the core of the software, which performs the tumour growth calculation based on the DGE model. This was developed with Visual C++.

Figure 3.7 shows the main procedure of tumour growth calculation. First, at each time increment, the module calculates the number of newborn tumour cells in each voxel using Double Gompertzian-exponential growth. Second, it calculates the number of tumour cells which leave each voxel and the number of tumour cells which spread into each voxel.

For asymmetric growth and spread, the module loads the coordinate data file of the tumour structures from disk. When the program performs the calculation, it searches every voxel and determines its spatially variant values such as K^{in} related to the specific tissue structures encompassed by the voxel.

After the calculation, the updated tumour cell density information is displayed by the module **Growth_Display**, which was written with MatrixVB.

The module **Growth_Display** provides a graphic display in various graphic formats, such as contour or isodensity surfaces of tumour cell density distribution on any slice or plane through the calculation matrix. Also, the tumour profile along specific directions and the spatial growth rate can be displayed by MatrixVB.

Figure 3.6 Flowchart of the Module ROSS_IO

Figure 3.7 Flowchart of the calculation module 3D_growth

CHAPTER 4

RESULTS

4.0 Overview of This Chapter

This chapter deals with the results of testing the ROSS software and investigating the behaviour of the DGE model. The tests and results are divided into three principal sections:

- Testing of the ROSS Software
- Testing and examining the behaviour of the DGE Model
- Comparison of the DGE model to *in vitro* and *in vivo* experimental and clinical data.

The overall purpose of these tests is to determine if the DGE tumour growth model can satisfy the requirements necessary for inclusion in the overall radiotherapy process model and adequately reflect tumour growth kinetics.

4.1 Software Tests

In order to examine if the ROSS software accurately reflects the DGE tumour growth model and determine the ranges of the critical parameters, the following tests were performed: the effect of the time increment Δt on the simulation results in terms of total number of tumour cells; the relationship among potential doubling time T_{pot} , time increment Δt and total growth time T ; and finally testing the geometric behaviour of the

predicted tumour profiles for bulk growth conditions. The behaviour of the model was tested under limiting conditions and the results are provided below.

4.1.1 The Impact of Time Increment Δt on the Results

The DGE tumour growth model is a numeric approximation of a first order non-linear differential equation. Therefore, the time increment Δt is a critical parameter in the software ROSS. Theoretically, the smaller Δt is, the more accurate the prediction will be. However, the smaller Δt , the greater the required time for computation. For such a three-dimensional model, it is not practical to choose an extremely small Δt . In order to examine the impact of Δt on the accuracy of tumour growth calculation, different Δt were chosen to simulate a realistic spheroid growth *in vitro*. These results were used to test the sensitivity of tumour growth (number of tumour cells produced after

Figure 4.1 The impact of Δt on the total number of cells.

some time t) on the value of Δt (Details of the growth conditions are provided in Section 4.3.2). It is desirable to know the impact of different Δt on the results in terms of the total number of cells in the whole tumour. For comparison, Δt is described as a fraction of the potential doubling time of the simulated tumour cells.

For this test Δt values ranging from $0.0005 T_{pot}$ to $0.2 T_{pot}$ were chosen and the remaining model parameters were fixed. The model was then used to calculate the total number of tumour cells over a growth time of 28 days for each Δt . The impact of Δt (represented as the fraction of T_{pot}) on the number of cells at day 28 is shown in Figure 4.1. As seen in this figure, the number of cells is almost invariant for values of Δt between $0.0005 T_{pot}$ and $0.005 T_{pot}$ and then starts to decrease with the increase of Δt . It is reasonable then to assume that the value predicted at $0.0005 T_{pot}$ is close to the value expected in the limit as Δt goes to zero. Referring to Figure 4.1 a choice of $\Delta t = 0.2 T_{pot}$ predicts the number of tumour cells to be only 72% of that with $\Delta t = 0.005 T_{pot}$, which is too large an error to be acceptable in the overall process model. However, choosing $\Delta t = 0.0005 T_{pot}$, requires about 120 minutes of computation time on a Pentium 200mhz with 64M ram. It was found that choosing $0.005 T_{pot} < \Delta t < 0.02 T_{pot}$ provides a reasonable choice, since the computational error is less than 2.5% and the computation time of approximately 10 minutes.

4.1.2 Effect of T_{pot} , Δt and Total Growth Time

In ROSS, once a value for Δt is chosen and input, the total number of calculation loops N_l for determining total tumour growth is calculated as follows:

$$N_l = \frac{24T}{\Delta t} \quad (4.1)$$

where T is the total growth time in days and Δt is the time increment in hours. As demonstrated in Section 4.1.1, the optimal choice of the time increment Δt is a function of the potential doubling time T_{pot} . Recall that T , T_{pot} and Δt are input parameters for

ROSS. For any given set of T , Δt and T_{pot} , then the software should generate the same result as it would when run with values of $2T$, $2\Delta t$ and $2T_{pot}$.

ROSS was tested with the two sets of parameters listed in Table 4.1. The results show that ROSS generates the same number of tumour cells, 3.4×10^8 for both sets of parameters, consistent with expectation.

Table 4.1 Parameters for the test of the relationship among growth time T , time increment Δt and potential doubling time T_{pot}

Test	T (days)	Δt (hours)	T_{pot} (hours)	N_{max}	N_g	$N_{maxvoxel}$	N_{gvoxel}	Voxel size	Predicted # of cells
1	300	2	72	2.7×10^{10}	5×10^6	1×10^6	1×10^3	1 mm ³	3.4×10^8
2	150	1	36	2.7×10^{10}	5×10^6	1×10^6	1×10^3	1 mm ³	3.4×10^8

4.1.3 Symmetry Tests

The DGE model assumes that under ideal conditions a tumour will grow as a perfect sphere. Therefore, at any time, the tumour cell density distribution should be symmetric along any straight line passing through the origin (location of the seed tumour cell). In the model, as discussed in chapter 3, symmetric growth is achieved when the parameters K^{in} and $K^{spatial}$ are set to be spatially invariant in the software.

In order to test the symmetric behaviour of the simulated tumour, the tumour cell densities along the x, y and z axes, as well as along two diagonal directions, 45° and 135° to the z plane were extracted from a growth matrix calculated using the parameters set shown in Table 4.1. The profiles of the tumour cell densities corresponding to these directions are displayed in Figure 4.2. As can be seen in the figure, the profiles are symmetric. Tumour growth symmetry was tested further by selecting profiles not passing through the origin of tumour. Figure 4.3 shows tumour cell density profiles in 4 symmetric cross sections, each of which lies in a plane parallel to a plane passing through the centre but displaced by 5 mm. Again as expected it shows that ROSS and the DGE model produce symmetric tumours. As a final test of symmetry, a contour plot of tumour cell density in a plane passing through the tumour origin is shown in Figure 4.4.

Figure 4.2 Tumour profiles passing through origin

Figure 4.3 Tumour profiles 5 mm from the origin

As seen in this figure, the iso-lines of tumour cell density describe a circle. These three tests confirm that symmetric growth is achieved by selecting K^{in} and $K^{spatial}$ to be spatially invariant.

Figure 4.4 Contours of tumour cell density in a central slice for a simulated symmetrical tumour.

4.1.4 Limiting condition test

Before the implementation of the DGE model, it is necessary to test its behaviour under extreme conditions such as assigning maximum and minimum values for the critical parameters. Under these conditions one can predict the expected behaviour of the model and hence test if the model is coded as expected

The Δt test was described in Section 4.1.1. The parameters N_{max} , $N_{max\ voxel}$, and T_{pot} are considered to be inherent characteristics of individual tumours and hence their

effect is considered later in this chapter. The extreme condition tests in this section focus on the behaviour of the model as a function of the Gompertz number N_g ($0 \leq N_g \leq N_{\max}$) and N_{gvoxel} ($0 \leq N_{gvoxel} \leq N_{\max voxel}$).

Two extreme cases were tested for N_g and N_{gvoxel} at their minimum value. In the first, N_g was set to its minimum possible value, 0. The other variables were set as listed in Table 4.1. Results show that under these conditions the model does not produce any tumour, as would be reasonably expected because the tumour growth should be completely inhibited if the N_g is set to 0. Similarly, in the second, N_{gvoxel} was set 0, and the results indicate that there is no tumour growth under this condition. The zero growth in this case is because the tumour growth is fully prohibited in each voxel as would be expected in the DGE model.

The three other limit combinations of N_g and N_{gvoxel} were tested. In the first (“test 1”), Both N_g and N_{gvoxel} were set to their maximum possible value $N_{\max}$ and $N_{\max voxel}$ respectively. Both $N_{\max}$ and $N_{\max voxel}$ were set to 10^6 . This represents an ideal exponential growth without any systemic and local feedback inhibition until the tumour grows to a maximum limit and then all growth should cease. In the second (“test 2”), N_g was set to $N_{\max}$ (10^6) but N_{gvoxel} was set to $0.01N_{\max voxel}$ ($N_{\max voxel}=10^3$). This represents the situation of tumour growth where local feedback begins when a voxel contains more than 10 cells, but there is no systemic feedback inhibition until the tumour reaches its maximum size at which time all growth should stop. In the third (“test 3”), N_g was set to $0.01N_{\max}$ and N_{gvoxel} was set to $N_{\max voxel}$. This represents the situation where the tumour grows without local voxel feedback inhibition, but systemic feed back should begin where the overall tumour size is 10^4 cells. The results for these three tests are shown in Figure 4.5. As can be seen in this figure, for the “test 1” the tumour growth is exponential until the tumour reaches its maximum value 10^6 and then the tumour remains static. This behaviour is exactly what would be expected of the DGE model. In the DGE model under these conditions, the tumour is growing at it maximum rate which is defined by T_{pot} . In this case, the growth fraction is 1, i.e. all cells are dividing and there is no

cell loss during its development. In this case T_{pot} can be determined from the slope of the curve “test 1”. From Figure 4.5, it can be seen that the break point from exponential growth to Gompertzian growth occurs at 10 for the “test 2”. The only difference between “test 2” and “test 1” is that N_{gvoxel} , the local feedback inhibition parameter, has been turned on and set to influence growth when any voxel contains more than 10 cells. As shown in Figure 4.5, for “test 3” the growth retardation occurs at 10^4 . In this case there is no feedback inhibition at the voxel level, but the overall tumour size should begin to inhibit growth when there are more than 10000 cells. As can be seen the model behaves as expected. From these three tests on limit condition combinations of N_g and N_{gvoxel} , the coding of the model behaves as would be predicted. The DGE model predicts growth that is slower than exponential growth under normal conditions, and exponential growth is the limit growth for the DGE model under ideal tumour conditions.

Figure 4.5 Cell growth as a function of time

4.2 DGE Model Tests

Tests were done to investigate the behaviour of the DGE model. These include a comparison with another model under identical growth conditions, a demonstration that the model predicts a necrotic core surrounded by a rim of proliferating cells for a spheroid, the generation of local regional nodal involvement, and a test for the simulation of asymmetric growth. These tests were performed to assure that the DGE model will meet the requirements for inclusion in the overall radiotherapy process simulation.

4.2.1 Comparison with Virtual Radiotherapy

As discussed in Section 3.3, the DGE tumour growth model is extended from a single kinetic Gompertzian Exponential model. The single Gompertzian model is also the basis of the software Virtual Radiotherapy 2.0. Virtual Radiotherapy (VR 2.0) was

Figure 4.6 Comparison between Ross and Virtual Radiotherapy 2.0.

developed by Dr. David Wilkins of the Ottawa Regional Cancer Centre (ORCC) for evaluating the impact of various radiotherapy fractionation schemes on local tumour control. As a test, tumour growth as predicted by ROSS was compared to the predictions of VR 2.0.

For this comparison, the same biological conditions were simulated in the two models run on the same computer with the same operating system. First, in order to remove the spatial behaviour of the DGE model, the spread coefficient K^{out} was set to 0 and k^{in} was made spatially invariant. This was required since VR is a non-spatial bulk growth model. By adjusting K^{out} and k^{in} as defined above, DGE will only allow tumour cells to grow in a single voxel and no cells are allowed to migrate out from this voxel. Second, N_g was set to N_{max} , so that systemic feedback does not play a role. Finally in this simulation, the voxel size in DGE was set large enough to contain 10^5 cells.

Under these conditions, the DGE model should reduce to the single Gompex model. In the simulation, T_{pot} was chosen to be 96 hours (a reasonable potential doubling time for human tumours), and N_{gvaxel} was set to be 10^3 for both ROSS and VR 2.0. Both simulations started with a single cell which was allowed to grow for 10,000 hours. The results given in Figure 4.6 show that the two simulations agree, it must point out, for most points, the results by both simulation are exact same.

4.2.2 Tumour growth profiles

The DGE tumour growth model is a spatial model, and as such can predict cell density distributions in a tumour. For a symmetric tumour, spatial behaviour can be described by the profiles passing through the origin of the tumour. In the DGE model, three parameters can affect the shape of tumour profiles: the spread coefficient K^{out} , and the local and global Gompertzian trigger numbers N_g and N_{gvaxel} . When performing the profile tests, two sets of values for the model parameters were chosen, and are listed in Table 4.2. The first set of parameters was chosen to simulate a slowly spreading tumour ($K^{out} = 0.1$) and the second one represents a fast spreading tumour ($K^{out} = 1.0$). Figure 4.7 is a plot of the tumour cell density showing a tumour cell density profile through the

centre of each of the two simulated tumours. As can be seen in this figure, the highest concentration of tumour cells is at the centre of the tumour for both cases. For slow spreading, the TCD at the tumour centre approaches $N_{\max \text{ voxel}}$. This means that number of tumour cells has almost reached its maximum in the central voxel before the tumour begins to spread to adjacent voxels. In contrast, for the rapidly spreading tumour ($K^{\text{out}} = 1.0$) the tumour has spread to many surrounding voxels by the time the tumour cell density in the central voxel has reached only 65% of $N_{\max \text{ voxel}}$, resulting in a radius 4 mm greater than the slow spreading tumour.

Table 4.2 Different combinations of N_g , K^{out} and $N_{g\text{voxel}}$ for profile tests

Test	T (days)	Δt (hours)	T_{pot} (hours)	$N_{\max}$	N_g	$N_{\max \text{ voxel}}$	$N_{g\text{voxel}}$	Voxel Size	K^{out}
1	300	2	72	5×10^8	5×10^8	1×10^6	1×10^5	1 mm ³	0.1
2	Same	Same	same	Same	5×10^5	Same	1×10^4	Same	1

Figure 4.7 Tumour profiles passing through the centre for different spread coefficients.

4.2.3 Normalized Spatial Growth Rate

The DGE tumour growth model can dynamically predict the number of tumour cells in each voxel, and therefore offers the opportunity to calculate the spatial variation in growth rate within the tumour. The relative growth rate in each voxel describes the net growth rate of cells during each time increment Δt , which is given by:

$$\lambda_{i,j,k} = \frac{N_{i,j,k,t+\Delta t} - N_{i,j,k,t}}{N_{i,j,k,t} \Delta t} \quad (4.2)$$

The growth rate profile through a tumour can provide very useful information relating to potential radiosensitivity. Using the same parameter set as described in the first column of Table 4.1, the rate of tumour growth in each voxel was calculated after the tumour had

Figure 4.8 normalized growth rate passing through the centre of tumour

been allowed to grow for 844 hours. This was then normalized to 100 relative to the voxel with the largest growth rate. The relative growth rate (“growth rate”) and tumour cell density (“TCD”) are shown in Figure 4.8. From this figure, it can be clearly seen that the rate of growth is almost zero in the central voxel of the tumour where the tumour cell density is at its maximum. In contrast, the highest growth rate appears at the periphery of this simulated tumour. The growth rate sharply decreases moving from the edge of the tumour towards the centre. This is consistent with the behaviour observed in spheroid and some solid tumours; that is, there is a dormant (necrotic) core and a viable rim of tumour cells within the true tumour^[62]. This behaviour will be discussed further in Section 4.3.3 and Section 5.4.

4.2.4 Test for Asymmetric Growth

As mentioned in Sections 3.2 and 3.3, in the DGE model the variable $k_{i,j,k}^{in}$ has been defined as the ability to accept new cells from other adjacent voxels, given by:

$$k_{i,j,k}^{in} = k_{i,j,k}^{spatial} \frac{N_{i,j,k,t}}{N_{\max \text{ voxel}}} \quad (4.3)$$

where $k_{i,j,k}^{spatial}$ is a spatially variant coefficient when asymmetric growth is simulated.

When performing the test of asymmetric growth, the voxels were divided into two classes; for normal host tissues a relatively low value 0.1 of $k_{i,j,k}^{spatial}$ is given while a larger value of 1.0 is assigned to voxels such as those containing lymphatic drainage where tumour spread is more likely. The model first calculates the number of tumour cells to move out of a voxel and then distributes these cells to the adjacent voxels. The software checks each adjacent voxel and determines its $k_{i,j,k}^{spatial}$. After each cycle Δt the new cells destined to migrate out are distributed to the adjacent voxels preferentially by $k_{i,j,k}^{spatial}$. This asymmetric growth was tested using the parameters given in the first column of Table 4.1 for a growth time of 300 days. The results are shown in Figure 4.9. Figure 4.9(a) illustrates a predefined structure where the voxels in red were given a high value for $k_{i,j,k}^{spatial}$ and Figure 4.9 (b) is an iso TCD plot in a plane passing through the origin. It

can be seen that after the tumour has been allowed to grow there is preferential spread to voxels with a large $k_{i,j,k}^{spatial}$.

Figure 4.9 Asymmetric growth predicted by the DGE model

(a) a predefined tissue structure where the colored voxels are regions of enhanced ability for tumour cell migration. (b) is an image of iso tumour cell density passing through the origin.

4.2.5 Local regional nodal involvement

In the DGE model we define a separate non-contiguous spread function to mimic local regional seeding. It is assumed that this seeding is governed by a low probability in each voxel. Local regional spread is spatially variant where the relative probability of spread to a non-adjacent voxel after each Δt is given as:

$$P_{i,j,k}^{nodal} = k_{i,j,k}^{nodal} \frac{\sum \Delta N_{l,m,n}}{N_{max}} \quad (4.4)$$

where $k_{i,j,k}^{nodal}$ is the spatially variant constant of probability of seeding to voxel (i, j, k) . We also assumed that the probability of local seeding depends on the size of the tumour, which is represented by $N_t = \sum_{i,j,k} N_{i,j,k}$. N_t is the total number of cells within the tumour at time t .

To test the behaviour of local seeding in this model, a spatial invariant value of 1.0 % was assigned to $k_{i,j,k}^{nodal}$ for several voxels. During the calculation ROSS checks each voxel after each time step. If a voxel is empty, a random number is then generated and is compared with $p_{i,j,k}^{nodal}$. When the random number is less than the nodal spread probability $P_{i,j,k}^{nodal}$, this empty voxel would be given a seed tumour cell and non-contiguous seeding is achieved. Figure 4.10 shows a simulation of local discontinuous seeding. The primary tumour is at the centre of the slice and is surrounded by four secondary tumours.

Figure 4.10 Iso map of the tumour with local seeding (the scale is in mm)

4.3 Comparison of the DGE Model with Existing Experimental and Clinical Data

The DGE tumour growth model was validated with two spheroid experiments which were reported by Wallace^[63] and Freyer^[64, 65] separately. The DGE model was also used to simulate the growth of a virtually untreated Glioblastoma multiforme (GBM) tumour and compared to data as reported by Kansal et al^[45]. The procedure of the validation is described in this section.

4.3.1 Spheroid Growth *in Vitro*

It is very difficult to validate the three-dimensional tumour growth model due to the shortage of complete spatial tumour growth data in both humans and animals. The spheroid model is an *in vitro* approximation to the complicated biological process of tumour growth and has been developed as a useful tool for testing tumour growth and response to various therapies in a controllable environment^[56, 65-68].

(a)

(b)

Figure 4.11 Illustration of a spheroid, (a), an image of an 800 μm spheroid, reprint from reference 25, (b), the illustration of the central cross section of a spheroid

The behaviour of spheroid growth *in vitro* can mimic that of solid tumour growth *in vivo*. For the past thirty years many efforts ^[56, 64] have been made to investigate the behaviour of spheroid tumour growth *in vitro* and then deduce the dynamic tumour behaviour *in vivo*. In these experiments the necessary nutrients are supplied to the cancer cells from the surrounding growth medium in a constant and uniform manner. As the spheroid grows, cells in different locations within the spheroid have different nutrient and environmental conditions due to the ability of these molecules to migrate through the spheroid ^[69, 70]. Cells in the inner part of the spheroid are often located beyond the critical diffusion distance from the periphery and die or go to G_0 from lack of nutrients. This is also observed clinically in that solid tumours are characterized by the development of central necrosis as they increase in size. Figure 4.11 (a) is a photograph of typical spheroid taken by Dr. R. M. Sutherland. Figure 4.11 (b) is a diagrammatic cross section of a fully developed spheroid.

4.3.2 Ovarian Spheroid Data Validation

The DGE tumour growth model was compared to experimental data for the spheroids grown from the A2780 human ovarian cancer cell line. These data were provided by Dr. Julia Wallace of the Physics Department of Carleton University.

Table 4.3 summarizes the measurements of the ovarian spheroids extracted from J. Wallace's Ph.D. thesis. Please refer to the reference [63] for the details of the experiments. The diameter of A2780 spheroid as a function of growth time is shown in Figure 4.13.

Table 4.3 Summary of the measurement of ovarian cell line ^[63].

Cell Diameter (μM)	Cell Density (Cells/ mm^3)	Exponential Doubling Time (Hours), Mono Layer
14.7	Tissue Like	17.3

In order to fit the experimental data of A2780 spheroid growth previously mentioned, the parameters are estimated for the DGE model as follows. The voxel size was chosen to be $100\ \mu\text{m} \times 100\ \mu\text{m} \times 100\ \mu\text{m}$ based on the maximum diameter $804\ \mu\text{m}$. The choice of voxel size is discussed in Chapter 5. The Gompertzian trigger value for the voxel level, N_{gvoxel} , was chosen to be 0.01 times the maximum packing density of the spheroid for the best fit to the experimental data. Because the size of the spheroid is too small (less than $1000\ \mu\text{m}$ in diameter), it was assumed that there was no systemic retardation and therefore $N_g = N_{\text{max}}$. The measured initial cell doubling time for monolayer growth (optimally perfused) for the A2780 cell line was used for T_{pot} in this simulation. The values of the other parameters used in the simulation are listed in Table 4.4.

Table 4.4 Parameter set for the simulation of A2780 ovarian spheroid

k^{out}	N_{gvoxel}	N_{max}	N_g / N_{max}	Voxel Size	T_{pot}
0.1	10	27,000,000	90%	$0.001\ \text{mm}^3$	17.3 h

In this simulation, there were initially 200 A2780 ovarian cells placed in the central voxel in a three-dimensional matrix at time $t = 0$. By applying the parameters in Table 4.4, the number of cells inside the spheroid was calculated and recorded as a function of growth time. Moreover, the diameter of the simulated spheroid was determined by measuring the iso map of tumour cell density over the central slice of the simulated spheroid at the times of 10, 14, 21, 28, 35 days respectively. Figure 4.12 shows the comparison of the spheroid diameters from this simulation to the experimental measurement of Wallace^[63].

In the simulation of the A2780 Ovarian spheroid growth *in vitro*, a high global Gompertzian trigger number N_g was chosen to remove the systemic influence. This is because the spheroid is very small (diameter = $804\ \mu\text{m}$) in Wallace's experiment, and growth was observed to be approximately exponential. Therefore, compared with a fully

developed spheroid (diameter is about 1100~1500 μm), the size of A2780 spheroids is less than the saturation volume of a spheroid (900~1100 μm in diameter) [64, 67]. Considering that the DGE model is a discrete model, it may overestimate the diameter of spheroid. However, the maximum variation is less than 100 μm , which is the voxel size of the simulation. The χ^2 value was calculated to be 3.15 for this fit based on the uncertainty of the measurement and model prediction. Since there are 2 parameters in the DGE model, so the degree of freedom is 3 for this fit and the ratio of χ^2 to number of freedom is 1.05, which is close to 1. The χ^2 test showed that the DGE model fitted the wallce' measurement with the chosen parameters.

Although the DGE model fit the ovarian spheroid growth data very well (see Figure 4.12), the systemic impact was not reflected in this validation due to the insufficient spheroid measurement data in this experiment. In order to validate the DGE model further, other measurement data are examined below.

Figure 4.12 The increase in diameter of A2780 ovarian cells spheroid with time from measurement and simulation.

4.3.3 Comparison of the DGE Model to the MCC 26 Spheroid Growth Data

The DGE model was compared to the experimental data for the mouse MCC26 spheroid ^[64], which was reported by James P. Freyer. In his paper, he presented the correlation relationship among the maximum size, necrosis and the growth rate.

In his experiments, spheroids 140-160 μm in diameter containing 400-600 cells were placed into a culture medium with a fully homogenous and consistent nutrient supply which was maintained during the development of spheroids. The spheroid growth was measured in volume and in total cell content as a function of growth time. For details of this experiment, please refer to reference ^[64]. The measurement results of MCC 26 cell lines are summarized in Table 4.5.

Table 4.5 The measured parameters for spheroid growth by JP Freyer ^[64]

Cell Line	Mono Layer Initial Doubling Time (hours)	Spheroid Initial doubling Time (hours)	Spheroid Max. Size μm^3	N_{max}	Viable Rim (μM)	Cell Packing ($\text{C}/\mu\text{M}^3$)
Mouse Mcc26	13	24	3.2×10^{10}	2.4×10^6	257	2.2×10^{-4}

Based on Freyer's ^[64] measurements and observations of the spheroid growth, the parameters for this simulation were chosen as follows. The maximum number of cells within the spheroid and the packing density were chosen to be 2.4×10^6 and $220,000 \text{ mm}^{-3}$ respectively, as observed by Freyer. Since the maximum diameter of the spheroids in this work was approximately 1,500 μm , voxels of $100 \mu\text{m} \times 100 \mu\text{m} \times 100 \mu\text{m}$ were chosen for the simulation with the DGE model. For such a small voxel, it is assumed that cell loss occurs when the number of cells in each voxel exceeds 22.5% of the maximum packing density ($N_{\text{max voxel}}$). This corresponds to 55 cells per voxel and was a best fit to the experimental data.

Freyer's measurements showed that the systemic feedback inhibition of tumour growth occurs when the cell number in the spheroid reached between 1% and 10% of the

maximum tumour size (burden) $N_{\max}$. In this simulation, a systemic Gompertzian trigger number $N_g = 0.04N_{\max}$ was chosen as a best fit to the experimental data.

The potential doubling time of the MCC26 spheroids was not reported in Freyer's paper. However he determined the initial cell doubling time in the same cell lines with an initial experiment for exponential growth data for monolayer and spheroid. The initial doubling times were determined to be 13 hours and 24 hours for monolayer and spheroid respectively. It was believed that the doubling time for monolayer growth more closely represents the true potential doubling time of cells than spheroid growth data. This is because even for small spheroids the nutrient supply is more sufficient in monolayer culture than in the spheroid growth. The difference of doubling time between spheroid and monolayer growth will be discussed in Section 5.6. The parameters used in the DGE simulation are summarized in Table 4.6.

Table 4.6 The parameters used in the simulation for spheroid growth.

$N_{\max \text{ Voxel}}$	$N_{\text{growth}} / N_{\max \text{ spheroid}}$	$N_g / N_{\max}$	$N_{\max}$	Δt (hours)	T_{pot} (hours)
224000	0.225	0.04	2.4×10^6	0.1	13

The simulation of the MCC26 spheroid growth started from 400 cells at the centre of the matrix, which is consistent with Freyer's experiments. In this simulation, the total cell count, the growth rate along a line passing through the centre of the spheroid, and the radius of the spheroid were recorded every 3 days over the 31.5 days of the experiment.

Figure 4.13 shows the total number of cells in the spheroid as a function of growth time for both this simulation and Freyer's measurement. As seen in Figure 4.13, the simulation agrees with the measurement quite well, although the number of cells determined in the simulation was less than that of measurement. It is suspected that this is because Freyer deduced the number of cells from a measurement of spheroid volume and packing density, which could lead to an overestimate of cell number in spheroid. This is further discussed in Section 5.3.

It is difficult to provide a meaningful quantitative analysis of fit of the DGE model to experimental because the uncertainties in both the spheroid and human data are unknown. The DGE model is primarily intended to be used to understand the population trend over a short time scale and in this sense it is more meaningful to perform a sensitivity analysis of the model. T_{pot} is a biologically measured quantity. It does not affect the TCD distribution other than modifying the time scale and hence does not warrant sensitivity analysis. Similarly the maximum cell density at the voxel level is a constant and again is not investigated.

The parameters which were subjected to sensitivity analysis were N_{max} , N_g , k^{out} , N_{gvaxel} and Voxel Size. The model was then run with a $\pm 10\%$ variation in each of these parameters while the remaining parameters were fixed at the values given in table 4.6

In this analysis a sensitivity parameter S_d is used and is defined as:

$$S_d = \frac{\Delta N / N}{\Delta V / V} \quad (4.5)$$

where, ΔN is the variation of the value of the function as a result of a change ΔV in a parameter. In our case ΔN is the change in the calculated number of tumour cells after 35 days of tumour growth. It should be noted that since the function describing cell growth is non linear and has multiple independent variables the sensitivity analysis is only valid for the conditions stated. In this case, conditions typical of spheroid growth of MCC26 cells *in vitro* after 35 days of growth. Under these conditions:

$S_d = 0.75$ for N_{max}

$S_d = 0.25$ for N_g

$S_d = 1.05$ for k^{out}

$S_d = 1.0$ for N_{gvaxel}

$S_d = 2.15$ for voxel size.

In general the larger S_d , the more sensitive the calculation is to errors in the estimate of the parameter.

There are two methods to estimate the volume in order to compare Freyer's results with the DGE simulation. In the first method, the volume of simulated spheroid was estimated from the total number of tumour cells within the spheroid and the cell packing density of the spheroid, as defined in the following equation ("prediction 1"):

$$V = \frac{N}{\rho_{pd}} \quad (4.6)$$

where, ρ_{pd} is the packing density of tumour cells (mm^{-3}), N is the number of cells within the simulated spheroid and V is the estimated volume of spheroid at growth time. However this is not always true. When the tumour cell density (or packing density) of the spheroid is not uniformly distributed, the actual volume could be bigger than the prediction obtained from Eq.4.6. This is discussed further in chapter 5.

Figure 4.13 Number of cells as a function of growth time.

An alternate method of determining spheroid volume in the DGE model is by measuring the radius of the simulated spheroid from the TCD profile through the centre of the spheroid as shown in Figure 4.8 and then deduce the volume of spheroid at each time of interest. This is the method used in “prediction 2”. Figure 4.14 shows the volume of the spheroid as a function of growth time for both measurement and the prediction using the above two methods. As can be seen, the measurement does not agree well with “prediction 2”, especially after the saturation. However, it is more accurate to estimate the spheroid volume by the radius of simulated spheroid than by cell number-volume conversion.

Figure 4.14 Volume growth as a function of growth time

Volumetric doubling time (or population doubling time) is one of the important parameters to determine the stage of a tumour and in the development of a treatment plan for a patient. This is very difficult to measure *in vivo*. The DGE tumour growth model is

able to predict the population doubling time of the spheroid. In the estimation, assuming that the population growth at each time interval is linear, then the population doubling time is defined as:

$$T_d = \frac{0.693(T_2 - T_1)}{\ln \frac{V_2}{V_1}} \approx \frac{0.693(T_2 - T_1)}{\ln \frac{N_2}{N_1}} \quad (4.7)$$

where, N_2 and N_1 are the cell populations at time T_2 and T_1 respectively.

Figure 4.15 Volume doubling time as a function of time.

Figure 4.15 shows the volumetric doubling time as a function of growth time for the simulated spheroid in Freyer's experiments. As seen in this figure, the doubling time of the spheroid becomes longer with the increase in growth time (and hence tumour size).

This is usually attributed to a smaller growth fraction and cell loss increases due to the lack of nutrients.

The necrotic fraction is defined as the fraction of the tumour volume occupied by necrotic cells. The DGE model cannot directly distinguish necrotic cells, but these can be estimated by using the relationship between the growth rate and the behaviour of necrotic cells.

Because the growth rate within the necrotic region of a tumour is very small, the simulated spheroid can be divided into two shells according to the spatial growth rate defined in Section 4.2.3. These two shells are the core of necrotic cells and the rim of viable cells.

Figure 4.16 Necrosis fraction as a function of growth time

It was assumed in the simulation that the voxels with a growth rate below 10% of the maximum growth rate constituted the necrotic area. Therefore, the “necrotic fraction” in the DGE model can be predicted approximately by the following equation:

$$f_n = \left(\frac{r_n}{r_t}\right)^3 \times 100\% , \quad (4.8)$$

r_t and r_n are the radius of spheroid and the necrotic core respectively. Eq.4.8 is based on the assumption that the spheroid and the necrotic core are perfect spheres.

Figure 4.16 is a plot showing the necrotic fraction as a function of the growth time for the simulated spheroid. It shows that there was no necrotic zone very early in the spheroid growth. Necrosis development started from the 10th day and increased very rapidly to 38% of the volume and continued to increase with total growth time.

Many experiments^[64, 65] have been done to investigate how the necrotic region forms and develops. It is believed that there is a direct relationship between necrosis and environment conditions, such as pH, nutrient supply and oxygen etc. Many investigators^[64, 65] have concluded that necrosis forms because the tumour destroys the normal vasculature and hence oxygen and nutrients cannot sufficiently diffuse to the central part of the tumour, which results in the inhibition of cell growth and cell death.

Figure 4.17 Normalized spatial growth rate for EMT6 spheroid and a simulated MCC26 spheroid.

The growth rate is spatially distributed within a spheroid. Understanding how the growth rate changes at the rim of the viable sphere helps us to understand the diffusion gradient of oxygen or nutrient distribution within a spheroid.

Freyer ^[62] reported his measurement of spatial growth rate variation for EMT6 spheroids. The thickness of the viable rim was measured to be 0.251 mm for the spheroid with a diameter of 1.5 mm. The normalized spatial growth rate is shown in Figure 4.17. These experiments were simulated in ROSS with the DGE model using cell data for MCC26 cells since accurate data was not available for EMT6 cells.

The relative growth rate of the simulated MCC26 spheroid was also normalized to 1 for the comparison. Both the measured data set of EMT6 spheroid from Freyer and the predicted curve from this simulation are shown in Figure 4.17. The growth rate decreases with the distance from the outmost periphery to the centre. As seen in Figure 4.17, the maximum growth rate is at the outmost edge of the simulated spheroid. The growth rate approaches zero when the position of the voxel is far from the tumour surface, which agrees well with experiment. There exists an apparent difference between two curves, which may be due the cell line differences and a weakness in the model. This is discussed in Section 5.2.

4.3.4 Glioblastoma Multiforme Tumour Growth Simulation

Glioblastoma multiforme (GBM) is the subject of much research because it is one of the most malignant brain tumours ^[71, 72] and represents almost 30% of all primary brain tumours. Surgery alone rarely controls GBM because the tumour cells spread throughout the whole brain ^[25]. To increase the possibility to kill the remaining tumour cells, radiation therapy usually follows surgery although success is rare.

A simulation of GBM using the DGE growth model was compared to some clinical data available from several untreated GBM tumours. For the details of these data, please refer to the references ^[45, 59, 71, 72].

The results compared in this study include the total number of cells within a GBM tumour, the necrotic fraction and the volumetric doubling time, all as a function of

growth time. These parameters were selected since they are useful in determining a tumour's malignancy, prognosis for its future growth, and the selection of candidate treatment plans.

It is almost impossible to obtain continuous growth data for untreated GBM tumours, but it is possible to find tumour growth data at specific time points such as spheroid (which can be obtained from *in vitro* measurement), minimal detectable lesion size, size at diagnosis and size at death for different patients. Table 4.7 summarizes the measurement data extracted from the medical literature. For the GBM tumours, total cell numbers and volumetric doubling time measurements, including growth and necrotic fractions for a virtual patient, were reported in Reference 45.

Table 4.7 measurement data for virtual untreated GBM tumour.

	SPHEROID	DETECTABLE LESION	DIAGNOSIS	DEATH
Radius	0.5mm	5 mm	18.5 mm	25 mm
Cell number	10^6	10^9	5×10^{10}	10^{11}
Growth fraction	36%	30%	20%	9%
Necrotic fraction	46%	49%	55%	60%
Volume-doubling time	6 days	45 days	70 days	105 days

The tumour in the virtual patient modelled with DGE was generated using parameters extracted from the literature [59, 71, 72]. Based on these reports, the average survival time of these patients from diagnosis to death was calculated to be 3.25 months and the survival time from onset of disease to death was estimated to be 18.2 months^[72]. A time scale is now added to the non-continuous events in Table 4.7 for this virtual patient. It was assumed that the first brain tumour cell occurs at time 0. It would be diagnosed 15 months later and the brain tumour would result in the death of the virtual patient after another three months. In addition, the cell number, growth fraction etc. of GBM spheroid were acquired in these literature. The two times “detectable lesion” and “diagnosis” are defined as the minimum detectable lesion size by imaging modalities of the time and the average tumour size at the time of diagnosis, ie. When the patient became symptomatic for their disease.

Table 4.8 lists the parameters used in the simulation of the untreated brain tumour. The GBM tumour was simulated from a very small volume of 0.125 mm^3 to the very large volume 65 cm^3 . Since the maximum matrix size for ROSS is $30 \times 30 \times 30$, the voxel size was chosen to be 8 mm^3 in this simulation. It was also assumed that when the

Table 4.8 The parameters used in the simulation for GBM brain tumour growth

$N_{\text{max voxel}}$	$N_{\text{growth}} / N_{\text{max voxel}}$	N_s / N_{max}	N_{max}	T_{pot}	Voxel Size	Δt
2×10^6	0.0005	0.2	2.2×10^{11}	96h	8 mm^3	2h

total number of tumour cells reaches 20% of N_{max} as reported by Kansal ^[45], systemic feedback starts to take effect. In addition, the local inhibition feedback occurs when the number of tumour cells in a local voxel was 0.05% of the maximum packing density ($N_{\text{max voxel}}$). Finally the maximum number of tumour cells was chosen to be 2.2×10^{11} , which is equivalent to a tumour with a radius ^[45] of 37.5 mm.

The total number of cells within the GBM tumour as a function of growth time for both measurement and simulation are shown in Figure 4.18. Please note that the tumour reaches the volume of minimal detectable lesion ($r_t = 5 \text{ mm}$) five months after onset of the tumour. It then reaches the volume ($r_t = 18.5 \text{ mm}$) that can be diagnosed after another 7.7 months. Finally the virtual patient dies 3.7 months after diagnosis with a tumour volume of 65 cm^3 .

As mentioned in Section 4.3.3, the necrotic fraction is one of the useful parameters to describe the stage of tumour development.

In this simulation, the spatial growth rate was calculated using Eq.4.2. For a symmetric tumour, the profile of growth rate passing through the tumour origin was calculated. The necrotic radius was estimated from this profile and the necrotic fraction was calculated using Eq.4.7.

The simulated necrotic fraction and the experimental measurement with time are shown in Figure 4.19. As can be seen, the prediction of necrotic fraction fits well with

the experimental measurement at the last two data points, but underestimates the necrotic fraction early in the growth of the tumour. The prediction is about 30% lower than that of the first measurement point. There may be many reasons for this, including the fact that a large voxel of 8 mm^3 was chosen in this simulation, but initially the tumour was very small (less than 1 mm^3) and the DGE model cannot provide any spatial information for such a small tumour with a large voxel. This limitation could be removed by modelling on a more powerful computer and using a larger calculation matrix.

Figure 4.18 Plot of the total number of cells within the tumour as a function of growth time (Note: the solid curve corresponds to the simulation using the parameters listed in Table 4.8, and the points are the data derived from the medical literature. The data were measured for different patients and at different times for the tumour development, i.e., they were isolated events.)

The volumetric doubling time is the time to double the tumour volume. Many experiments have shown that the volume doubling time is close to the cell cycle time at very early stages. With increasing tumour volume, the growth fraction decreases, and cell loss rate increases, which results in the retardation of overall observed tumour growth and hence an increase in volume doubling time.

In our simulation, it is assumed that the GBM tumour grew spherically. Therefore the radius of tumour, r_{Tumor} , could be estimated by the tumour profile passing through the tumour origin. The volume of the simulated tumour, V_{tumor} , was calculated as

$$V_{tumor} = \frac{4}{3} \pi r_{tumor}^3 \quad (4.9)$$

The volumetric doubling time was estimated with Eq.4.8.

Figure 4.20 shows the volumetric doubling time of this virtual untreated GBM tumour. The dotted points represent the volumetric doubling time at the time of spheroid with 1 mm^3 , lesion with 1 cm^3 , diagnosis and death, while the solid curve represents the doubling time predicted with our DGE model.

Figure 4.19 Necrosis fraction as a function of growth time.

Figure 4.20 Volumetric doubling time change with growth time.

CHAPTER 5

DISCUSSION

5.0 Overview of this Chapter

In Chapter 3, a Double Gompertzian-Exponential (DGE) tumour model was developed, which is an expansion of the single Gompertzian exponential growth model. The DGE tumour model differs from its parent model in several fundamental ways: It has a spatial character and hence it provides the opportunity to introduce spatially variant tumour characteristics such as growth rate and spread probabilities and it can allow tumour growth to respond to locally variant micro environmental conditions. The DGE model also introduces the ability to model asymmetric growth, as well as local and distant metastasis. In Chapter 4 it was shown that the software ROSS is an accurate coding of the DGE model. Moreover, it was demonstrated how the various parameters of the model affect its behaviour. The software was then used to model several experimental and clinical systems. The results have shown that simulation with the DGE tumour growth model agrees well with observed spheroid growth data and with compiled data describing a virtually untreated brain tumour.

In this chapter, Section 5.1 discusses the tests on how the choice of Δt affects the results of calculation. It also addresses the observations on how the choice of voxel size in the model affects the accuracy. Section 5.2 discusses how the DGE model incorporates local and systemic feedback into the overall tumour growth kinetics. Section 5.3 looks at the tumour cell density profiles predicted by the DGE model and its

clinical implications. Section 5.4 examines the spatially variant growth rate which is a fundamental property of the DGE model and section 5.5 discusses the ability of the DGE model to simulate asymmetric growth. Section 5.6 is a discussion of cell doubling time and its importance in the DGE model. In section 5.7 the ability of the DGE model to simulate local seeding and the importance of local seeding in the determination of the total tumour burden is discussed. Section 5.8 examines the effect of waiting time on the patient outcome and section 5.9 describes how this model can be linked with the spatial radiobiological cell killing models to investigate new approaches to the calculation of Tumour Control Probability (TCP). Finally, Section 5.10 summarises the advantages and disadvantages of this model.

5.1 Effect of Δt and Voxel Size

Since the DGE tumour growth model is an approximation of an ODE, the accuracy of the model is affected by the choice of the time increment Δt (as demonstrated in Section 4.1.1.) and the voxel size as shown in section 4.3. In general the smaller the value of Δt , the better the accuracy. Due to insufficient computational power available for this project, it was impractical to choose a very small Δt . As an example, consider a human tumour with a potential doubling time of 96 hours. Depending on tumour type, metastatic potential etc. the time scale for modeling from the induction of the tumour to death in an untreated patient would be from 3 to 10 years. As mentioned in Section 4.1, the choice of Δt equal to $0.0005 T_{pot}$ can generate a good accuracy, but in this example would require approximately 10^6 time iterations for the life span of the tumour. For the full 3D tumour model with a $30 \times 30 \times 30$ matrix it would require approximately 10 hrs of computer time on the existing hardware.

Voxel size is also a critical factor which affects the accuracy of the model. In the DGE model, cell growth in each voxel is independent of cell growth in other voxels. Each voxel has its own growth kinetics, but these growth kinetics are a function of voxel size. If the voxel size is too large, then the model cannot address biologically important parameters such as heterogeneity of the tumour, variations in growth rate, tumour cell density etc. A voxel of linear dimension between 0.1 mm and 0.5 mm is optimal. This

corresponds to the maximum distance that nutrients can diffuse from the edge to the centre of the voxel ^[25]. Our tests in Section 4.3.3 have shown that with a voxel volume of 0.001 mm^3 , the DGE model fit the measured spheroid growth data very well. The current maximum matrix size is $30 \times 30 \times 30$ in the software ROSS. This was chosen based on the speed and memory limitations of the computer used for simulations. Assuming that the optimal voxel dimension is 0.1 mm in linear dimension then with a $30 \times 30 \times 30$ matrix, DGE can only simulate a tumour of up to 1.5 mm radius. This is approximately the limit at which a tumour can be detected with most imaging systems. In section 4.3.4 it was shown that this matrix size is too small to simulate a large brain tumour which can be in excess of 65 cm^3 . For such a tumour a matrix of approximately $400 \times 400 \times 400$ would be required. This would represent approximately 2500 more calculation points than in the existing system.

In summary it was found that the CPU speed and memory were major limitations to running the DGE software to simulate clinical situations. The system used for the simulations was a Pentium 200 MHz with 64M RAM memory. With a modern PC many of these limitations can be reduced and a more realistic choice for Δt and matrix size can be run in a reasonable time period.

5.2 Effect of local and systemic feedback

In Section 4.1.4 it has shown that the DGE model could exhibit both local and systemic feedback effects. This feature is essential to model different types of tumours since it is known that tumours are heterogeneous and that different feedback mechanisms come into play in different parts of the tumour.

The mechanism of systemic feedback inhibition during tumour development results from a number of factors, which are summarized as follows.

First, biological experiments ^[73, 74] have shown that the interstitial pressure increases inside the core of a tumour with increase in its volume. This pressure makes it hard to transport the necessary nutrients and oxygen into the core of the tumour and transport the waste out, resulting in tumour growth retardation. Second, tumours must develop their own vasculature system in order to support their growth. But these

vasculatures are usually abnormally developed. The vessel systems within the tumour may block each other and restrain the nutrient transport to some areas. The situation becomes more serious with an increase in tumour volume ^[75]. Third, as the tumour outgrows its vascular system, rapid cell proliferation near capillaries will push other cells into regions remote from a blood supply, where there is an inadequate concentration of oxygen and other nutrients and leads to a progressively enlarging necrotic zone, resulting in cell loss ^[25, 53]. Finally, apoptosis, metastasis, immunologic attack and exfoliation also contribute to the growth retardation.

Generally the systemic feedback inhibition is described by N_g , and is the break point from the exponential growth to Gompertzian growth. Referring to the Section 4.1.4 and Figure 4.5, the systemic feedback comes into effect when the number of cells is between N_g and N_{max} . It is thought that there exists a maximum patient burden for tumour growth, based on observations of the existence of a maximal tumour burden from many animal studies^[76-78]. The maximum tumour burden is when the tumour kills the host and cannot acquire nutrients. In the DGE model, the patient's tumour burden is associated with the maximum number of tumour cells N_{max} , which is the number of cells that a patient body can support whether the cells are in a primary bulk tumour, or local seeding or metastasis. N_{max} varies from patient to patient and host status, as well as the type of tumour and tumour biology. Systemic feedback in this model is mainly associated with growth fraction and tumour burden N_{max} . As one of the three critical parameters for tumour growth, κ_{gr} (growth fraction) is the ratio of the number of cells in a cycling state to the total number of cells within the tumour. For example if κ_{gr} is 0.8, 80% of the cells within the tumour are in their cycling state and the remaining 20% are in the quiescent state. Since the cell-cycle kinetic effects are not included in the DGE model at present, it is not easy to distinguish the contribution of feedback of growth fraction at the local and systemic levels. It is well accepted that hormones and other tumour growth factors ^[25, 75] should be included as part of both the systemic feedback and the local feedback. Since these factors can affect the growth fraction of the tumour, part of the growth fraction can be included in the system feedback inhibition, and part of the growth fraction can be included in local feedback inhibition. The growth fraction at the

systemic level is the a weighted average of the growth fraction over the entire tumour. The use of average growth fraction can overestimate the growth rate at locations away from the tumour surface as shown in Figure 4.17. This is because growth fraction is spatially variant within the tumour. The growth fraction at the outmost periphery is close to 1.0 due to the availability of sufficient nutrients and then decreases to near zero as the point of interest moves toward the centre of the tumour.^[64] This is one of the shortcomings of this model. In the future, cell-cycle kinetics should be included in the next version of the DGE model which may overcome this weakness.

Referring to Figure 4.8, the DGE model can predict the growth rate profile for a symmetric tumour. The high growth rate is at the periphery of the tumour and the centre of the tumour is dormant. This behaviour is similar to that observed in spheroids (*in vitro*) and in some human tumours. The formation of a dormant core within a real tumour is due to many factors. A principal factor is that the micro-environmental conditions (pH, oxygen and other nutrient and growth factors) cannot support cell growth. Most of the cells in the dormant core are either dead or in the quiescent state G_0 . If cells are in G_0 , it is possible for them to convert to a cycling state if conditions allow. In the DGE model, N_{gvoxel} is the only parameter used to describe tumour growth feedback in a local voxel. This is the condensation of all biological and micro-environmental factors such as pH, oxygen and blood vessels distribution etc. ^[25, 53]. The formation of the dormant core predicted by this model is due to relatively independent growth kinetics for different voxels. Although this model can provide useful information such as tumour cell density profiles, it cannot distinguish between quiescent G_0 and dead cells. This is one of the shortcomings of this model.

In the DGE model it was assumed that the micro-environmental conditions are homogenous across the whole tumour, and therefore a spatially invariant N_{gvoxel} is used. However, the micro-environmental conditions are non-homogenous for most tumours. Modern imaging technologies such as MRI and PET are capable of determining the distribution of some of the micro-environmental conditions such as pH and blood flow so that a spatially variant N_{gvoxel} might be determined using these functional imaging systems. If the parameter N_{gvoxel} is set to be spatial variant to reflect the non-

homogenous environmental conditions, for example pH distribution, the DGE tumour growth model could be used to investigate the effect of pH on the growth behaviour of human tumours.

5.3 Discussion of Tumour Profile

The DGE tumour growth model can predict non-homogenous tumour cell density as described in Section 4.2.2 and the tumour profiles in Figure 4.7. The ability to predict tumour profile is one of the important features of this model and may be applicable to clinical radiotherapy.

The DGE model has some significant differences from its parent model, the single kinetics GompEx growth model. The single kinetics GompEx tumour growth model, a widely accepted model in cancer therapy, assumes that tumour growth is spherically symmetric and that the cell density (packing density) is homogenous in the tumour. However the packing density is heterogeneously distributed within tumours as shown in Section 4.1.3 and 4.3.3. Thus using a single GompEx model to predict the growth in cell number and then deducing the volume using a single (maximum) packing density can underestimate the tumour volume. This has been demonstrated in Figure 4.14. Conversely, using the single GompEx model to predict the volume growth and then calculating the total number of tumour cells can overestimate the total number of tumour cells. Both total cell count and tumour volume are used to determine the dose and the radiation treatment fields limits, hence the conversion between cell count and volume can lead to either a reduction in TCP or an increase in NTCP. Since the DGE model is a spatial model, it can predict tumour cell density distribution information such as tumour cell density profiles as presented in Section 4.1.3. These profiles avoid the overestimation in total cell number or underestimation in tumour volume. The prediction of tumour cell density profiles and tumour cell growth rate profiles using the DGE model also suggested that a spatially variant dose over the tumour is required in response to these non-homogenous distributions. That is, a high dose should be delivered to voxels with a high cell density and with low growth rates.

Tumour profiles have been presented in Section 4.2.2 for two different spread coefficients which demonstrate that tumour profiles can be changed by altering the spread coefficients. Clinically it is known that different tumours spread at different rates and have different inter tumour cell densities. These range from dense solid tumours such as sarcomas with a high packing ratio to more diffuse tumours such as those found in the mediastinum with a low packing ratio. In the DGE model, the profile of a tumour is dominated by the parameter k^{out} , which is defined as the ability of cells in one voxel to spread to an adjacent voxel. The parameter k^{out} is analogous to the Fick Diffusion coefficient^[59] in a diffusion model. In the DGE model, k^{out} is a unitless variable ranging from 0.0 to 1.0. The selection of k^{out} mainly depends on the voxel size, tumour type, and local tissue architecture. Given a fixed voxel size, a large k^{out} represents fast spreading cells. As an example consider Section 4.3.4, in which a GBM was modelled. This is known to be an aggressive fast spreading tumour and in this case a value of 1 was assigned to k^{out} for the simulation.

Allowing the parameter k^{out} to be spatially variant in this model would simulate asymmetric tumour growth as is discussed in more detail in Section 5.5. A spatially variant k^{out} is particularly useful in the simulation of tumour growth in a region containing different structures. For example, if a tumour grows in an area which includes bone, normal tissue, blood vessels and lymphatic drainage, the rate of spread within the volume might be spatially variant. The voxels with blood vessel and lymphatics may have an increased spread rate, the voxels containing normal tissue would have an average spread rate and there may be a low spread rate in voxels containing bone. A spatially variant spread rate within the tumour would result in asymmetric growth. Asymmetric growth is very important in treatment planning and to some extent affects the decision about the radiation field size and shape. Moreover, a tumour with asymmetric growth would have a higher overall growth rate than a symmetric tumour due to its high surface to volume ratio and hence an increased ability to distribute nutrients. Therefore a spatially variant k^{out} might help to understand the behaviour of asymmetric tumour growth and improve radiotherapy strategy.

As mentioned in Chapter 2, many spatial models such as the diffusion-interaction models ^[45, 57-59] suggested that the spread of tumour cells could be described by a diffusion equation over relatively short times. These models assume that the diffusion of tumour cells results from mechanical confinement pressure. The concept of “diffusion” in these spatial tumour growth models oversimplifies tumour development in biological systems. For example the matrix supporting tumour cell growth as well as the diffusion coefficients are invariant everywhere in the simulated area ^[59]. These models do not have the ability to simulate features such as a tissue bone interface which is critical in cancer therapy. The DGE model introduces the factor k^{out} to overcome this problem. This is discussed in more detailed in Section 5.6.

5.4 Growth Rate

In Section 4.2.3, spatially variant tumour growth has been predicted by the DGE tumour model. As seen in Figure 4.8, the growth rate varies with location in the tumour. There is a higher growth rate at the periphery of the tumour and a lower growth rate at the tumour core, which is consistent with observed tumour development *in vivo*. Cells in the centre of a tumour are either dead or in G_0 state. Cells in G_0 state are very radio-resistant but are not growing. When the DGE tumour growth model is introduced into a spatial radiobiological model, it will provide the opportunity to introduce spatially variant cell kill rates over the tumour. Higher kill rates, or increased radiosensitivity, would occur at the rim of the tumour if it is highly proliferative ^[25, 53]. This will be an extremely important advance in radiobiological modelling, particularly for fractionated radiotherapy. In each fraction, the outermost rim of tumour is preferentially killed due to its higher radiosensitivity than that in the centre. As a result the tumour will “shrink” from the outside towards the middle. As this shrinkage occurs the outermost part of the necrotic core will be re-oxygenized and start to grow again changing its radiobiological dynamics. Effectively the outer tumour cells of the necrotic zone will move into mitosis from G_0 state. This advantage is one of the important reasons that the fractionation technique has been widely used in radiotherapy, but it has never been accurately introduced into any radiobiological models. Through the marriage of the DGE model

with a radiobiological model it will be possible in the future to investigate the effect of variable fraction size and the time between fractions for different tumours.

5.5 Discussion on Asymmetric Growth

The DGE model can simulate both symmetric and asymmetric growth, as has been demonstrated on Section 4.2.4 and 4.2.5. The parameter k^{in} is a spatially variant variable in the DGE model and directs the degree of asymmetric growth. The variable k^{in} defines the ability of one voxel to accept cells from an adjacent voxel. The determination of k^{in} depends on the tissue type and architecture of tumour and a spatially variant k^{in} allows the tumour to grow more rapidly in one direction than another. This behaviour was demonstrated in Section 4.2.4. Clinically this is the norm rather than the exception. Most solid tumours and of course all diffuse tumours are not spheres. For example breast tumours exhibit dendritic growth^[79] and prostate tumours preferentially spread to the seminal vesicles^[80]. In brain tumours this is thought to be an even bigger problem. The low cure rate for brain tumours is not only due to their rapid growth but also to the fact that long before the lesion can be diagnosed, microscopic tentacles or local seeding have already invaded the surrounding brain parenchyma. The consequence is that surgery usually fails due to the local seeding, and since brain tumours are extremely radio-resistant, it is difficult to get a large enough dose to the entire tumour without doing too much normal tissue damage. Hence the current simulation of asymmetric growth with the DGE model may help to understand how these structures including tissue bone interface, blood vessel and lymph structures affect the tumour growth and further improve the strategy of radiotherapy in these patterns.

5.6 Discussion on Potential Doubling Time

T_{pot} , the potential doubling time of tumour cells, is the only true biological parameter used in this model to describe the tumour growth. It is the shortest possible doubling time of the tumour under perfect growth conditions. However, the tumour

generally has an overall effective doubling time that is much longer than the true T_{pot} as has been shown in Figure 4.15 and Section 4.3.3. The DGE tumour growth model is an expansion of a single kinetic Gompertzian-Exponential model. There are some significant differences between the two models which are discussed as follows:

In many references [49, 50, 53], the biological meaning of the slope of a single GompEx model at its initial exponential phase is interpreted as the potential doubling time of tumour cells. However it is very difficult to find exponential growth data for human *in vivo* tumours. In most cases tumours are observed while in their Gompertzian growth phase and the calculation of the true potential doubling time using a single GompEx model can overestimate this value.

An example of this was shown in Section 4.3.3. The population doubling time of the spheroid was found to be 24 hours when modelled with a single kinetic model by Freyer [64]. Additionally, the authors determined that for the same cell line the population doubling time was 13 hours for idealized monolayer growth. This was tested using the DGE model and the results are shown below in Figure 5.1. As can be seen using the

Figure 5.1 Growth curve for different potential doubling times

shortest measured T_{pot} provided a good fit to the measured spheroid data. It must be stressed that within the DGE model, the T_{pot} must in fact be the absolute minimum doubling time for cell growth. All other effects within the model then cause the overall or local tumour growth rate to decrease. Note however, that a doubling time of 24 hours when used in a single kinetics Gompertzian model fit the spheroid growth data very well. But this is not the true T_{pot} , but rather falls out of the single Gompertzian model because this model cannot simulate a spatially variant growth rate. Since the single kinetics GompEx model has been widely used to explain the biological characteristics of tumours, we should be very cautious in interpreting data determined by fitting to this empirical model. The same is not necessarily true for the DGE tumour growth model. It was assumed that initial tumour growth is exponential in each local voxel and thus at any time t there is in general a spatial variation in growth rate as each voxel enters into Gompertzian growth. In this aspect, the Double kinetics GompEx tumour growth is more realistic than the single kinetics GompEx model. In many cases, the exponential model is used to estimate the tumour growth with a population doubling time (or doubling time) in a very short time scale (less than 100 days), but in this case the volume doubling time must change with the stage of tumour as seen in figure 4.15 and Figure 4.20. This can result in a significant error if a T_{pot} determined from a single exponential model is extrapolated to other times of tumour growth. The DGE model will provide a time invariant T_{pot} which can avoid the risk of inappropriate choice of volumetric doubling time. This is an important feature of the DGE model.

5.7 Discussion of Local Seeding

In Section 4.3.4, a virtual untreated brain tumour was simulated with the DGE model. In the simulation, it was assumed that the GBM brain tumour is a bulk tumour in a homogenous medium. This assumption can underestimate the tumour growth. As mentioned previously, the local regional seeding is one of the most critical factors to affect the cure rate of brain tumours and it was reported that local seeding occurs in a short time after the onset of a brain tumour^[45]. In order to reflect this difference, let us

assume two situations of a tumour growing from two initial cells. In one case there are two brain tumour cells within a patient both in the same voxel or effectively a bulk growth single Gompex condition. In the other case there are two cells at two different locations inside an organ, effectively the DGE case. Figure 5.2 shows the ratio of the total cell number predicted by the DGE model for both cases. This clearly shows that early in tumour development the growth is same for both bulk growth and the case of double seeding. The total tumour size begins to differ when the number of cells in the original voxel exceed the local Gompertzian trigger number. The overall growth then slows down, but in the condition where the two cells originated in different voxels, overall growth is still in exponential phase. This is an excellent demonstration of the fact that the DGE model can simulate different growth conditions as a result of it's spatial character. In the second case, once the two separate tumours merge or the total number of tumour cells exceeds the systemic trigger level, the growth fraction decreases and the growth for the two conditions begins to converge and the ratio of the two growth rates approaches 1. The timing of this depends on the distance between the 2 seed voxels. When the two seeds are very close, say, adjacent voxels, there would not be a large difference between the single and double voxel seeding. Figure 4.18 shows the ratio of number of tumour cells for two cases as a function of growth time. The time of diagnosis occurs at a time of 9600 hours post onset as mentioned in Section 4.3.4 and the ratio for both cases is 1.85 at that time. It will require a higher overall tumour dose to achieve the same TCP control rate, in the case of double seeding with respect to single seeding. If the same dose is prescribed to both cases, the control rate would be lower for the tumour with double seeding. For a real brain tumour, the number, locations and time of seeding is very difficult to predict, but it is thought that once tumour seeding occurs, patients are at a higher risk.

Figure 5.2 The ratio N_{DGE} / N_{Bulk} versus growth time
 N_{DGE} and N_{Bulk} are the cell numbers predicted with DGE model and Bulk model respectively

5.8 Waiting Time Assessment

It has been suggested that the waiting time between cancer diagnosis and treatment could affect the local control probability^[40]. It is possible that the DGE model can be used to assess the impact of waiting time on the tumour control probability without resorting to crude estimates of population doubling time. Consider a brain tumour with 5×10^{10} cells (26.5 cm^3) at the time of diagnosis and further assume that the shape of the brain tumour is a sphere. A dose D is delivered to decrease the number of remaining tumour cells to N . Then, the tumour control probability is given as:

$$TCP = \exp(-QN(t)) \quad (5.1)$$

where Q is the surviving fraction after delivering dose D to the tumour.

Mackillop^[40] has suggested that the tumour is in exponential growth during the waiting time from diagnosis to treatment:

$$N(t) = N_0 \exp(0.693t / T_d) \quad (5.2)$$

where N_0 is the initial number of tumour cells at diagnosis and T_d is the population doubling time of the tumour. But it is very difficult to measure the tumour growth and then deduce the population doubling time from clinical observation. However, for such a brain tumour, it was reported that the population doubling time of tumour at the time of diagnosis is 70 days^[45]. In order to show the impact of the waiting time on the TCP, two methods were applied.

Assume that the prescribed dose, D , can achieve an initial TCP of 80% at the time of diagnosis. The relationship between TCP and waiting time can be given by the following equation:

$$TCP = 0.8^{\frac{N(t)}{N_0}} \quad (5.3)$$

The TCP was calculated for different waiting times with Eq. 5.2 and Eq. 5.3 and is shown for the exponential case in Figure 5.3 and labelled as “Exponential”.

The impact of the waiting time on TCP was also estimated with the DGE model. In this simulation, the total number of cells $N(t)$ was simulated with the parameters listed in Table 4.8 for waiting times of 0, 10, 20, 30, 40 and 50 days. TCP was then estimated with Eq. 5.3. The result is also shown in Figure 5.3 and labelled as “DGE”. As shown in the Figure 5.3, a delay of 50 days before treatment of a brain tumour could reduce the local control rate by approximately 10% without considering the increase in tumour volume which may extend beyond the treatment field. The estimation with the DGE model is somewhat lower than with the exponential model. But the advantage of the estimation with the DGE model is that the effective population doubling time, is not needed, only the true T_{pot} which can be measured in the laboratory.

Figure 5.3 TCP as a function of waiting time.

The choice of a brain tumour may not be the best example to show the effect of waiting time on treatment outcome, since the local seeding is thought to have usually occurred at the time of diagnosis and therefore the impact of waiting time on TCP is small compared to the effect of local seeding. However it is well documented that wait times can affect outcome in head and neck cancer ^[40]. In this case it is likely that the effect of waiting time may be underestimated. This is because the possibility of local seeding was ignored during the waiting time from the diagnosis to the radiation treatment and the tumour was treated as a bulk tumour with an effective doubling time. In the DGE model, the asymmetric character of head neck tumours will result in a greater overall growth. In addition, the DGE model can predict the probability of local or distant seeding occurring during the wait time. The probability of local seeding is proportional to the number of cells in the tumour. For some tumours e.g. head and neck cancer, once local and distant seeding occur in the patient's body, it cannot be cured by local

radiotherapy. Therefore, if all these factors are taken into account in the DGE model, the control probability could be appropriately estimated.

The DGE model may be a very powerful tool in assessing the effect of waiting times. Ontario is presently suffering from long waiting times and the government is struggling with this problem by trying to expand its facilities. This is a longer term solution and it cannot solve the waiting time problem faced today. This model can provide an opportunity to simulate the effect of waiting time on the outcome for different tumours and provide some useful suggestions to relieve the most critical aspects of the problem without extra radiotherapy resources. Based on the TCP degradation and the possibility of local and distant seeding, this model may provide a justification for the optimum management of radiotherapy resources. For example by applying this model to calculate the decrease in TCP for different tumours as a function of waiting time a least damaging scenario could be proposed. Tumours such as those of the head and neck cancer that are sensitive to the waiting time should be scheduled to be treated as soon as possible and those tumours such as prostate that are known to have very long T_{pot} times and are not as sensitive to waiting time can be postponed if there are insufficient radiotherapy resources.

5.9 NTCP/TCP and Spatial DGE Model

Currently, the most useful quantitative endpoints used to assess the outcome of cancer treatment by external radiotherapy are tumour control probability (TCP) ^[4, 16] and Normal Tissue Complication Probability (NTCP)^[14]. They condense spatial dose distribution data to yield a single number for each structure of interest e.g. an organ or a tumour. Unfortunately, NTCP/TCP endpoints are based upon rather simple models normalized to clinical endpoints and the links between the TCP/NTCP endpoints and survival rate are generally not well established. In some cases, the absolute value of the calculations does not agree with clinical experience as discussed in Section 1.4. However, it is reasonable to assume that even if TCP/NTCP values calculated by a model are not identical with the real clinical values, they can still be used to evaluate various candidate treatment plans in radiotherapy.

A variety of new approaches to calculate NTCP and TCP are currently under development to overcome the weakness of the present TCP/NTCP methods. Most of those TCP/NTCP models have focused on the heterogeneity of tumours. This approach requires spatial tumour growth information within a tumour, which cannot be provided by a dynamic tumour growth model such as exponential or Gompertzian growth models. The DGE model provides an opportunity to simulate tumour growth temporally and spatially. The spatial characteristic of the model has the potential to be applied to the TCP and NTCP calculation. In this approach, it is assumed that the tumour target can be outlined on imaging systems in which tumour cell density can be represented at the voxel level. Furthermore, current three-dimensional treatment plan systems provide voxel based dose distributions over the tumour target. It is now possible to combine spatial tumour density and spatial dose distribution to define the TCP as follows:

$$TCP = e^{-\iiint \rho_c(x,y,z) \omega_{sf}(x,y,z) dx dy dz}, \quad (5.4)$$

where $\rho_c(x,y,z)$ is the cell density within the tumour, which can be predicted by the DGE tumour growth model; $\omega_{sf}(x,y,z)$ is the spatial survival fraction of initial tumour cells at point (x,y,z) after external radiotherapy $D(x,y,z)$, which is the dose delivered to the point (x,y,z) . The key point of Eq. 5-4 is its spatial characteristic and this definition overcomes the idealized homogenous assumptions for tumour cell distribution and dose distribution. For example, using the linear-quadratic (L-Q) model, the surviving fraction at point (x,y,z) can be calculated as follows:

$$\omega_{sf}(x,y,z) = e^{-\alpha D(x,y,z) - \beta D^2(x,y,z)}, \quad (5.5)$$

where, α, β are the two coefficients of the L-Q model.

B. Sanchez-Nieto ^[81] suggested a TCP model which assumes that TCP depends on the spatial dose distribution. He introduced a Voxel-control-probability (VCP) to the TCP calculation, which is the probability of voxel tumour control. For a given treatment,

the VCP distribution over the tumour is generated, and the probability of local tumour control is defined as:

$$TCP = \prod_j VCP_j \quad , \quad (5.6)$$

where, VCP_j is the voxel-control-probability in voxel j .

The spatial behaviour of the DGE model provides an opportunity to realize this approach. Since the endpoint of the entire radiotherapy process model is NTCP/TCP, the link between the DGE tumour growth model and the spatial radiobiological model will help to understand the weak correlation between TCP/NTCP protocol and survival rate and then form a standard to assess the effect of technological change or process change on patient outcome. The DGE model may also be possibly linked with the fractionated dose distribution to investigate the impact of setup error and organ motion on the TCP. If the spatially variant individual tumour data such as pH, oxygenation, growth rate etc. are incorporated into the DGE model, this model can also perform outcome assessment on an individual patient basis when functional imaging becomes better (PET, MRI) and characteristics of individual tumours can be determined dynamically.

Most TCP/NTCP models are based on bulk tumour information and details such as local regional seeding and distant metastasis are not included. Since the DGE model can simulate asymmetric growth and local seeding, it provides a possibility to include this spatial information in the TCP model. The incorporation of tumour shape, local seeding and metastasis may increase the correlation between TCP and the observed clinical control rate of cancer treatment.

Currently there is very little data available to actually populate this tempo-spatial tumour growth model. The experimental data used to validate the model in Chapter 4 are either spheroid data *in vitro* or the average of data of a virtually untreated human tumour. The model can be used for individual patients if the spatial tumour cell information, the environmental conditions and the dependence of the model parameters upon these factors can be determined. It is believed that this model will be most useful when applied large patient populations.

5.10 Advantages And Disadvantages

The advantages and disadvantages of the Double kinetics Gompertzian-Exponential model are summarized as follows.

5.10.1 Advantages

The unique feature of the DGE tumour growth model is its spatial behaviour that is extended from an empirical model. It is also the first attempt to develop a spatial tumour growth based on an empirical model. Its advantages are described as follows:

The DGE tumour growth model overcomes the inherent disadvantage of the single kinetics Gompertzian-exponential tumour growth model which can only predict bulk tumour growth without spatial character.

Secondly, the DGE model uses five parameters to simulate the tumour growth. These parameters consist of patient burden, growth fraction of tumour, cell loss (or feedback inhibition) at the voxel level, spatially variant tumour spread dynamics, and the potential doubling time of tumour cells. This is a more sophisticated and reasonable model than the single kinetics GompEx model for predicting such a complicated biological system as tumour growth.

Thirdly, the temporal character of the DGE model provides an opportunity to investigate the impact of waiting time between diagnosis and treatment on patient outcome. It also might be applied to understand the tumour growth between two consecutive fractions in a course of radiotherapy and recommend the best treatment schedule for the cancer treatment.

Fourthly, the DGE model can predict spatial tumour cell density distributions and it provides an alternative way to calculate the tumour control probability (TCP). In addition, the spatial character of this model may potentially be used to optimize treatment plans.

This model can not only predict symmetric tumour growth such as seen *in vitro* but it can also be used to predict the asymmetric tumour growth such as at tissue interfaces which is observed clinically.

5.10.2 Disadvantages

The disadvantages of the DGE tumour growth model are summarized as follows.

At present the model uses a homogenous growth fraction over the entire tumour which can underestimate the growth rate at the periphery of the tumour after saturation.

Secondly, although this model is a spatial tumour growth model, it can only predict heterogeneity in cell number within the tumour. At present all factors which affect tumour growth are condensed into a single kinetic GompEx model at the voxel level, and as such the DGE model cannot predict the impact of spatial biological information such as pH, nutrient etc. on tumour growth. As has been shown this shortcoming can be easily removed from the model but the biological data will be very difficult to obtain.

Thirdly, the lack of spatial tumour growth data limits the understanding of the behaviour of the DGE tumour growth model. There is a significant shortage of tumour growth data, and most of what is available was published over twenty years ago. This might suffer from the large uncertainty due to the technological limitations of the time.

This model is a semi-empirical model. It is based on some of the fundamentally observed character of tumour growth but uses artificial parameters which are not necessarily fundamental to the system being modelled. This model will probably be more successful when applied to large populations rather than individual patients and care should be taken when trying to interpret the results for clinical use.

CHAPTER 6

CONCLUSIONS AND THE SCOPE FOR FUTURE WORK

6.1 Conclusions

Although an enormous amount of work has been done to improve the accuracy of dose delivery and significant changes have been made in radiotherapy equipment over the past three decades, the impact of these efforts on cancer control rates has been disappointing. A predictive model is being developed to investigate the entire radiotherapy process. This overall model includes all aspects of the radiotherapy process, not just dose delivery, and will include a radiobiological response model and a general tumour growth model.

A critical part of the entire radiotherapy process model is the Double kinetic Gompertzian Exponential (DGE) growth model, first suggested and developed by our group at the Ottawa Regional Cancer Centre. This model is extended from the single kinetic Gompertzian-exponential growth model. Our modifications introduce a spatial character to the growth model and explicitly address local and systemic feedback inhibitions, local feedback being at the voxel level and systemic feedback associated with patient tumour burden. Feedback at the voxel level can be considered as the condensation of all local biological factors which inhibit tumour growth such as interstitial pressure, lack of blood flow, the diffusion limit for oxygen, lowered pH, and cell loss due to apoptosis, exfoliation or immunologic attack.

The software ROSS has been developed using Visual C++, Visual Basic and MatrixVB under a Windows platform for the implementation of the DGE growth model. A series of tests have been performed to investigate the behaviour of the software ROSS and the DGE model. These tests, outlined in Section 4.1 and 4.2 have shown that the software ROSS reflects the DGE model quite well. The DGE tumour growth model has been tested against *in vivo* and *in vitro* tumour growth data extracted from the literature. These include A2780 Ovarian spheroid data, MCC26 spheroid growth data and a virtually untreated GBM brain tumour from the literature. The results of these tests are summarised as follows.

- The model is an approximation of the ODE equation and our tests have found that the reasonable range for time increment is $\Delta t \in [0.005T_{pot}, 0.02T_{pot}]$, which can generate a computational error less than 2.5% in an acceptable time. At present the calculation matrix of 30x30x30 limits the implementation of the model. Increased computer power will remove both of these limitations.
- The software was tested under limit conditions (maximum/minimum value for critical parameters in the model) and behaved as expected.
- The DGE model was compared to a simpler tumour growth model. The tests showed that under conditions where both models were valid the DGE model gave the same results as the single Gompertzian growth model.
- This model is a spatial tumour growth model. It predicts a spatially variant growth rate within a tumour such that in general there is a relatively dormant core and a rapidly growing periphery, as is observed in both human and experimental systems. The size, shape and growth rates of the viable rim and “necrotic” core are determined by the model parameters such as local Gompertzian trigger number, spread coefficient and tumour size.
- This model can simulate symmetric tumour growth as seen in spheroids grown *in-vitro* as well as asymmetric growth observed clinically at tissue interfaces. It can also simulate both local regional seeding such as nodal involvement as well as distant metastases.

- Both cell loss and a reduced growth fraction result in an observed tumour growth retardation. This is observed *in vivo* and *in vitro* and is predicted by the DGE model.

According to the DGE model, feedback inhibition at the local microenvironment level dominates the tumour growth early in tumour development. As the tumour gets larger, systemic feedback inhibition begins to dominate as the tumour volume exceeds the volume of the global Gompertz number.

The DGE model is a voxel based tumour growth model and as such it provides an opportunity to introduce spatial tumour information acquired as part of the patient staging process as the initial conditions for the growth model. It then provides a spatial framework within which to introduce radiobiologic models of tumour response to treatment and to investigate opportunities to improve the entire radiotherapy process. This model is a semi-empirical model as it is based on some underlying biological principals. It is important to remember that this model is not capable of predicting tumour growth in a specific individual but could be reflective of a large population.

6.2 Scope for Future Work

We have developed a unique tumour growth model for use in studying the radiation therapy process. The model has been shown to simulate spheroid growth and a virtually untreated brain tumour growth very well. However, there is still much more work to be done before this model can be considered complete.

A common weakness of the tempo-spatial tumour growth models is that there is very little untreated human tumour growth data to validate them. It is necessary to validate the model for specific human and animal disease sites and tumour growth.

To be clinically useful it is necessary to eventually acquire the spatial tumour density and metabolic data from conventional and functional imaging modalities, to interpret the data and to import it directly into the model.

There are five parameters used in the DGE tumour growth model. Only one of these, the potential doubling time of tumour cells, is a directly measurable biological factor. The other factors such as the local and global Gompertzian trigger number and

the spread coefficients are the condensation of myriad biological factors. It is essential to understand these parameters further and to characterize them for various tissue and tumour types. It would be meaningful to collect data and build a database for the Double kinetics GompEx tumour model. The collection and determination of the various parameters and their values for different tumours is very difficult.

Because the DGE model is one part of the entire radiotherapy process model, it is necessary to build an interface for the communication with other modules such as radiobiological response model being developed by other colleagues. I would suggest that the interface should consist of the three-dimensional tumour cell density and the parameters for tumour growth.

As mentioned in Chapter 5, a weakness of this model is that at present, limited data requires that we apply an average of the growth fraction in the DGE model. This can result in an overestimation of growth rate inside the tumour. Although the current DGE model can provide the tumour growth profiles, useful in cancer research, this cannot yet be directly associated with the radiosensitivity distribution within the tumour. Cell-cycle kinetics must be incorporated into the DGE model. This improvement of the DGE model would allow more accurate spatially growth rates to be determined based on systemic factors such as growth hormones and homeostatic control mechanism (HCM) and local variations in the micro environment. Cells in each voxel are in one of three states: dead, mitosis or quiescent. Overall growth rate and radiobiologic sensitivity will depend on the relative proportion of cells in each state. The introduction of cell cycle kinetics will allow the DGE model to predict not only the tumour growth profiles but also the distribution of cells in different states such as G_0 , M and death.

In the DGE model, the local feedback inhibition is described by only one variable N_{voxel} . This is the condensation of all local biological factors which affect tumour growth. By changing N_{voxel} to be a spatially variant parameter within the tumour, the model could reflect some of the non-homogenous environmental condition such as pH and eventually be used to investigate the effect of these parameters on the growth behaviour of human tumours.

Figure 6.1 graphically illustrates the development of a tumour over its lifetime. For convenience, we divide the history of tumour development into four periods. The first period is the time from the onset of disease to the time T_{vi} when tumour can be diagnosed. The second period is the waiting time from the time T_{vi} of tumour diagnosis to the treatment T_{pr} . The third period is the time during treatment. The last period is the possible recurrence period from the end of treatment to the time of death due to tumour recurrence. There exists a number of tumour cells, N_f , which is the critical number of cells in a tumour below which the patient is regarded to be free of disease. It is the objective of treatment to reduce the number of tumour cells below N_f .

Figure 6.1 A lifetime history of human tumour development

The DGE model, coupled with a radiobiologic model can help predict if a treatment is likely to reduce the number of cells below N_f .

The impact of the time delay ($T_{pr} - T_{vi}$) on tumour control probability has been discussed in Section 5.7. When the DGE model is coupled with a model such as VR, the probability of reducing the number of viable tumour cells below N_f can be estimated as a function of treatment delay.

The spatial behaviour of the DGE model provides a way to investigate treatment optimization. Currently there are three principal methods used in cancer treatment: surgery, radiotherapy and chemotherapy. It may be possible to extend the limits of the model to investigate which combinations of these modalities and in which order can generate the most efficient tumour cell killing and achieve the best outcome. The DGE model enables us to simulate complex physical-biological situations of cancer treatment. For example, tumour surgery can be simulated with the DGE model by setting the initial cell density to zero for the voxels in which the tumour was removed. Also, by using a radiobiological response model, the decrease of tumour cell density can be estimated spatially after radiotherapy and chemotherapy.

There is still significant work to be done on quantifying and defining the DGE model as well as integrating it with other models. It is hoped that the DGE model can eventually be a useful tool in modelling tumour systems and their response to various therapies.

References

- [1] Leunens, G., Menten, J., Weltens, C., Verstraete, J., and van der, S. E., Quality assessment of medical decision making in radiation oncology: variability in target volume delineation for brain tumours, *Radiother. Oncol.* 29:169-175 (1993).
- [2] Yamamoto, M., Nagata, Y., Okajima, K., Ishigaki, T., Murata, R., Mizowaki, T., Kokubo, M., and Hiraoka, M., Differences in target outline delineation from CT scans of brain tumours using different methods and different observers, *Radiother. Oncol.* 50:151-156 (1999).
- [3] Craig, T., Battista, J., Moiseenko, V., and Van Dyk, J., Considerations for the implementation of target volume protocols in radiation therapy, *Int. J. Radiat. Oncol. Biol. Phys.* 49:241-250 (2001).
- [4] A. Brahme, Dosimetric precision requirements in radiotherapy, *Acta Radiologica Oncology* 23:379-391 (1984).
- [5] Nutting, C. M., Khoo, V. S., Walker, V., McNair, H., Beardmore, C., Norman, A., and Dearnaley, D. P., A randomized study of the use of a customized immobilization system in the treatment of prostate cancer with conformal radiotherapy, *Radiother. Oncol.* 54:1-9 (2000).
- [6] H. Hans, Quality assurance in radiation therapy: physical aspects, *Int. J. Radiat. Oncol. Biol. Phys.* 10:59-65 (1983).
- [7] Goitein, M., Calculation of the uncertainty in the dose delivered during radiation therapy, *Med. Phys.* 12:608-612 (1985).
- [8] Fiorino, C., Set-up error for conformal radiotherapy of prostate cancer, *Radiother. Oncol.* 56:355-356 (2000).
- [9] Gerald Kutcher, Control, Correction, and modelling of setup errors and organ motion, *Semin. Radiat. Oncol.* 5:134-145 (1995).

- [10] Li, J. G. and Xing, L., Inverse planning incorporating organ motion, *Med. Phys.* 27:1573-1578 (2000).
- [11] Leong, J., Implementation of random positioning error in computerised radiation treatment planning systems as a result of fractionation, *Phys. Med. Biol.* 32:327-334 (1987).
- [12] Barthelemy-Brichant, N., Sabatier, J., Dewe, W., Albert, A., and Deneufbourg, J. M., Evaluation of frequency and type of errors detected by a computerized record and verify system during radiation treatment, *Radiother. Oncol.* 53:149-154 (1999).
- [13] Mijnheer, B. J., Battermann, J. J., and Wambersie, A., What degree of accuracy is required and can be achieved in photon and neutron therapy?, *Radiother. Oncol.* 8:237-252 (1987).
- [14] Dale, E., Hellebust, T. P., Skjonsberg, A., Hogberg, T., and Olsen, D. R., Modeling normal tissue complication probability from repetitive computed tomography scans during fractionated high-dose-rate brachytherapy and external beam radiotherapy of the uterine cervix, *Int. J. Radiat. Oncol. Biol. Phys.* 47:963-971 (2000).
- [15] Bartelink, H., Begg, A., Martin, J. C., van Dijk, M., van, '., V, van, d., V, and Verheij, M., Towards prediction and modulation of treatment response, *Radiother. Oncol.* 50:1-11 (1999).
- [16] H. Rodey, treatment volume and tissue tolerance, *Int. J. Radiat. Oncol. Biol. Phys.* 14:751-759 (1987).
- [17] Webb S, Optimum parameters in model for tumour control probability including inter-patient heterogeneity, *Phys. Med. Biol.* 39:1895-1914 (1994).
- [18] Webb S, the effect on tumour control probability of the setting of a multileaf collimator with respect to the planning target, *Phys. Med. Biol.* 38:653-666 (1993).
- [19] Jones, B. and Dale, R. G., Radiobiological modeling and clinical trials, *Int. J. Radiat. Oncol. Biol. Phys.* 48:259-265 (2000).

- [20] ICRU report 50 prescribing, recording, and reporting photon beam therapy, ICRU. Baltimore, (1993).
- [21] Johns Harold E. and Cunningham John, The physics of radiology, fourth edition, Charles C Thomas publisher, Springfield, USA (1983).
- [22] Khan, Faiz M., The physics of radiation therapy the second edition, Williams & Wilkins, Baltimore, USA (1994).
- [23] Van Dyk J., the modern technology of radiation oncology, medical physics publishing service, Madison, USA (1999).
- [24] Orton, C. G., Uses of therapeutic x rays in medicine, *Health Phys.* 69:662-676 (1995).
- [25] Holland F James, Cancer Medicine 5 edition, William Wilkins, Baltimore, USA (2000).
- [26] Purdy, J. A., Future directions in 3-D treatment planning and delivery: a physicist's perspective, *Int. J. Radiat. Oncol. Biol. Phys.* 46:3-6 (2000).
- [27] Schulz, R. J., Further improvements in dose distributions are unlikely to affect cure rates, *Med. Phys.* 26:1007-1009 (1999).
- [28] Wu, X., Zhu, Y., and Luo, L., Linear programming based on neural networks for radiotherapy treatment planning, *Phys. Med. Biol.* 45:719-728 (2000).
- [29] Willoughby, T. R., Starkschall, G., Janjan, N. A., and Rosen, I. I., Evaluation and scoring of radiotherapy treatment plans using an artificial neural network, *In Int. J. Radiat. Oncol. Biol. Phys.* 34:923-930 (1996).
- [30] Perez, C. A., The cost-benefit of 3D conformal radiation therapy compared with conventional techniques for patients with clinically localized prostate cancer, *Int. J. Radiat. Oncol. Biol. Phys.* 45:1103-1104 (1999).

- [31] Hayman, J. A., Lash, K. A., Tao, M. L., and Halman, M. A., A comparison of two methods for estimating the technical costs of external beam radiation therapy, *Int. J. Radiat. Oncol. Biol. Phys.* 47:461-467 (2000).
- [32] Borgelt, B. B. and Stone, C., Ambulatory patient classifications and the regressive nature of Medicare reform: is the reduction in outpatient health care reimbursement worth the price?, *Int. J. Radiat. Oncol. Biol. Phys.* 45:729-734 (1999).
- [33] Dunscombe, P., Samant, R., and Roberts, G., A cost-outcome analysis of adjuvant postmastectomy locoregional radiotherapy in premenopausal node-positive breast cancer patients, *Int. J. Radiat. Oncol. Biol. Phys.* 48:977-982 (2000).
- [34] Hermans, R., Van den, B. W., Rijnders, A., Doornaert, P., and Baert, A. L., Predicting the local outcome of glottic squamous cell carcinoma after definitive radiation therapy: value of computed tomography-determined tumour parameters, *Radiother. Oncol.* 50:39-46 (1999).
- [35] Reddy, S. P., Mohideen, N., Marra, S., and Marks, J. E., Effect of tumour bulk on local control and survival of patients with T1 glottic cancer, *Radiother. Oncol.* 47:161-166 (1998).
- [36] Canadian Cancer Statistics 2000, Canadian cancer foundation (2000).
- [37] Surveillance, Epidemiology, and End Results (SEER) Cancer Statistics Review 1973-1998, <http://www-seer.ims.nci.nih.gov/> (1999).
- [38] Rizwan, U. and Saeed, I. M., Structure and growth of tumours: the effect of Cartesian, cylindrical, and spherical geometries, *Ann. N. Y. Acad. Sci.* 858:127-136 (1998).
- [39] Lujan, A. E., Ten Haken, R. K., Larsen, E. W., and Balter, J. M., Quantization of setup uncertainties in 3-D dose calculations, *Med. Phys.* 26:2397-2402 (1999).
- [40] Mackillop, W. J., Fu, H., Quirt, C. F., Dixon, P., Brundage, M., and Zhou, Y., Waiting for radiotherapy in Ontario, *Int. J. Radiat. Oncol. Biol. Phys.* 30:221-228 (1994).

- [41] Zaider, M., Zelefsky, M. J., Lee, E. K., Zakian, K. L., Amols, H. I., Dyke, J., Cohen, G., Hu, Y., Endi, A. K., Chui, C., and Koutcher, J. A., Treatment planning for prostate implants using magnetic-resonance spectroscopy imaging, *Int. J. Radiat. Oncol. Biol. Phys.* 47:1085-1096 (2000).
- [42] Kelland, L. R. and Bingle, L., Radiosensitivity and characterisation of a newly established cell line from an epithelioid sarcoma, *Br.J.Cancer* 58:322-325 (1988).
- [43] Adam J.A. and Maggelkis S.A., Diffusion regulated growth characteristics of a spherical pre-vascular carcinoma, *Bulletin of math.Biology* 52:549-582 (1990).
- [44] Adam, J. A. and Maggelakis, S. A., Mathematical models of tumour growth. IV. Effects of a necrotic core, *Math.Biosci.* 97:121-136 (1989).
- [45] A. Kansal, R., Torquato, S., Harsh, G. R., IV, Chiocca, E. A., and Deisboeck, T. S., Simulated brain tumour growth dynamics using a three-dimensional cellular automaton, *J.Theor.Biol.* 203:367-382 (2000).
- [46] Antonio Bru, Super-Rough Dynamics on tumour growth, *Physics Review Letter* 81:4008-4011 (2001).
- [47] Sophia A., A modelling effects of Angioenic factors on tumour related Angiogenesis and viscularization, *Math.Models in Medical & Health Sciences* 247-268 (1998).
- [48] Sophia A., the effect of tumour growth factors on the growth rate of cell cultures, *Apply.Math.Letter* 5:53-56 (1992).
- [49] Laird, A. K., Dynamics of growth in tumours and in normal organisms, *Natl.Cancer Inst.Monogr* 30:15-28 (1969).
- [50] Norton, L., A Gompertzian model of human breast cancer growth, *Cancer Res.* 48:7067-7071 (1988).

- [51] Demicheli, R., Pratesi, G., and Foroni, R., The exponential-Gompertzian tumour growth model: data from six tumour cell lines in vitro and in vivo. Estimate of the transition point from exponential to Gompertzian growth and potential clinical implications, *Tumours* 77:189-195 (1991).
- [52] Kendal, W. S., The size distribution of human hematogenous metastases, *J.Theor.Biol.* 211:29-38 (2001).
- [53] E. J.Hall, Radiobiology for the radiologist, third edition, J. B. Lippincott company, Philadelphia (1988).
- [54] W. Duchting, Modelling of spatial cell growth, Modelling of Biomedical Systems, *IMACS* 19-24 (2001).
- [55] Ransom R., Computer in biology: An introduction, Open university press, Melton kenyes, England, 70-74 (1985).
- [56] Landry, J., Freyer, J. P., and Sutherland, R. M., A model for the growth of multicellular spheroids, *Cell Tissue Kinet.* 15:585-594 (1982).
- [57] Gatenby, R. A. and Gawlinski, E. T., A reaction-diffusion model of cancer invasion, *Cancer Res.* 56:5745-5753 (1996).
- [58] Gatenby, R. A., Models of tumour-host interaction as competing populations: implications for tumour biology and treatment, *J.Theor.Biol.* 176:447-455 (1995).
- [59] Burgess, P. K., Kulesa, P. M., Murray, J. D., and Alvord, E. C., Jr., The interaction of growth rates and diffusion coefficients in a three- dimensional mathematical model of gliomas, *J.Neuropathol.Exp.Neurol.* 56:704-713 (1997).
- [60] Cross, S. S. and Cotton, D. W., The fractal dimension may be a useful morphometric discriminant in histopathology, *J.Pathol.* 166:409-411 (1992).

- [61] Craciunescu, O. I., Das, S. K., and Clegg, S. T., Dynamic contrast-enhanced MRI and fractal characteristics of percolation clusters in two-dimensional tumour blood perfusion, *J.Biomech.Eng*121:480-486 (1999).
- [62] Freyer, J. P., Decreased mitochondrial function in quiescent cells isolated from multicellular tumour spheroids, *J.Cell Physiol*176:138-149 (1998).
- [63] Wallace J., Characterization of human ovarian cancer by magnetic resonance spectroscopy using in vitro and ex vivo models, Ph.D. Thesis, Carleton University, Ottawa, 95-102 (1996).
- [64] Freyer, J. P., Role of necrosis in regulating the growth saturation of multicellular spheroids, *Cancer Res.*48:2432-2439 (1988).
- [65] Freyer, J. P. and Sutherland, R. M., A reduction in the in situ rates of oxygen and glucose consumption of cells in EMT6/Ro spheroids during growth, *J.Cell Physiol*124:516-524 (1985).
- [66] Schwachofer, J. H., Crooijmans, R. P., van Gasteren, J. J., Hoogenhout, J., Jerusalem, C. R., Kal, H. B., and Theeuwes, A. G., Repair of sublethal damage in two human tumour cell lines grown as multicellular spheroids, *Int. J. Radiat. Oncol. Biol. Phys.* 17:591-595 (1989).
- [67] Freyer, J. P. and Sutherland, R. M., Regulation of growth saturation and development of necrosis in EMT6/Ro multicellular spheroids by the glucose and oxygen supply, *Cancer Res.*46:3504-3512 (1986).
- [68] Schwachofer, J. H., Crooijmans, R. P., Hoogenhout, J., Kal, H. B., and Theeuwes, A. G., Sublethal damage repair in two radioresistant human tumour cell lines irradiated as multicellular spheroids, *Tumour.Biol*12:207-216 (1991).
- [69] Buffa, F. M., West, C., Byrne, K., Moore, J. V., and Nahum, A. E., Radiation response and cure rate of human colon adenocarcinoma spheroids of different size: the significance of hypoxia on tumour control modelling, *Int. J. Radiat. Oncol. Biol. Phys.* 49:1109-1118 (2001).

- [70] Wheldon, T. E., Livingstone, A., Wilson, L., O'Donoghue, J., and Gregor, A., The radiosensitivity of human neuroblastoma cells estimated from regrowth curves of multicellular tumour spheroids, *Br.J.Radiol.*58:661-664 (1985).
- [71] Hoshino, T. and Wilson, C. B., Cell kinetic analyses of human malignant brain tumours (gliomas), *Cancer*44:956-962 (1979).
- [72] Yamashita, T., Fujii, S., Fujino, H., Mochimatsu, Y., Nakajima, F., Kyuma, Y., and Kuwabara, T., [Estimation of the growth rate of malignant gliomas by CT scanning (author's transl)], *Neurol.Med.Chir (Tokyo)*20:1095-1101 (1980).
- [73] Fingar, V. H., Wieman, T. J., and Doak, K. W., Changes in tumour interstitial pressure induced by photodynamic therapy, *Photochem.Photobiol.*53:763-768 (1991).
- [74] Baronzio, G., Freitas, I., Griffini, P., Bertone, V., Pacini, F., Mascaro, G., Razzini, E., and Gramaglia, A., Omega-3 fatty acids can improve radioresponse modifying tumour interstitial pressure, blood rheology and membrane peroxidability, *Anticancer Res.* 14:1145-1154 (1994).
- [75] Maggelkis S.A. and Adam J.A., Note on a diffusion model of tissue growth, *Apply.Math.Letter*3:27-31 (1990).
- [76] Rubio, N., Villacampa, M. M., El Hilali, N., and Blanco, J., Metastatic burden in nude mice organs measured using prostate tumour PC- 3 cells expressing the luciferase gene as a quantifiable tumour cell marker, *Prostate*44:133-143 (2000).
- [77] Gobbi, P. G., Ghirardelli, M. L., Solcia, M., Di Giulio, G., Merli, F., Tavecchia, L., Berte, R., Davini, O., Levis, A., Broglia, C., Maffe, G. C., Ilariucci, F., Dore, R., and Ascari, E., Image-aided estimate of tumour burden in Hodgkin's disease: evidence of its primary prognostic importance, *J.Clin.Oncol* 19:1388-1394 (2001).
- [78] Moore, A., Sergeyev, N., Bredow, S., and Weissleder, R., A model system to quantitate tumour burden in locoregional lymph nodes during cancer spread, *Invasion Metastasis*18:192-197 (1998).

- [79] Almand, B., Resser, J. R., Lindman, B., Nadaf, S., Clark, J. I., Kwon, E. D., Carbone, D. P., and Gabrilovich, D. I., Clinical significance of defective dendritic cell differentiation in cancer, *Clin. Cancer Res.* 6:1755-1766 (2000).

- [80] Koutrouvelis, P., Lailas, N., Hendricks, F., Gil-Montero, G., Sehn, J., and Katz, S., Three-dimensional computed tomography-guided monotherapeutic pararectal brachytherapy of prostate cancer with seminal vesicle invasion, *Radiother. Onc.* 60:31-35 (2001).

- [81] Sanchez-Nieto, B. and Nahum, A. E., The delta-TCP concept: a clinically useful measure of tumour control probability, *Int. J. Radiat. Oncol. Biol. Phys.* 44:369-380 (1999).