Growth of a virtual tumour using probabilistic methods of cell generation

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Abstract

A study into treatment enhancement in combined chemo-radiotherapy for unresectable head and neck cancer has initiated the development of a computer model of tumour growth. The model is based on biological parameters, and characterises tumour growth prior to chemo-radiotherapy. Tumour growth starting from a single stem cell is modelled using the Monte Carlo method. The type of the cell function, their relative proportions on mitosis, their proliferative capacity, the duration of the four phases of the cell cycle, the mean cell cycle time, and the cell loss due to natural causes are the main parameters of the basic model. A Gaussian distribution function operates in establishing the cell cycle time, with a mean value of 33 hours, while the cell type is sampled from a uniform distribution. With the established model, the sensitivity of the developed tumour's cell population to the stem, proliferative and nonproliferative ratio at mitosis was assessed. The present model accurately reflects the exponential distribution of cells along the cell cycle (70% cells in G1 phase, 15% in S, 10% in G2, 5% in M) of a developed tumour as described in the literature. The proportion of stem, finitely proliferating and resting cells during tumour growth is maintained within their biological limits (2% stem, 13% finitely proliferating, 85% nonproliferating cells). The ratio (R=3) between the time necessary to develop a clinically detectable tumour (10^9 cells) and the further time to grow to its lethal size (10^{12} cells) is in accordance with the biological data when tumour volume is compared for the two periods (30 doublings and 10 doublings respectively). In conclusion, computer simulation can illustrate the biological growth of a tumour and the cell distribution along the cell cycle. These distributions may then be used in the assessment of tumour response to radiotherapy and to specific chemotherapeutic agents.

Key words tumour growth model, cell cycle, stem cell, nonproliferating cell, cell distribution

Introduction

Head and neck cancer constitutes approximately 12% of all cancers. Over 95% are squamous cancers and 4-5% are salivary gland carcinomas or melanomas. With locally advanced head and neck cancer the relapse rate is 50-60% within 2 years and 20-30% develop distant metastases[1]. Therefore there is scope for improved outcomes of head and neck cancer treatment through consideration of biological responses to combined modality therapies. The current most commonly used treatment is combined chemo/radiotherapy with a single or multiple chemotherapeutic agents.

Pursuit of an enhanced treatment regimen is conventionally through a range of controlled clinical trials, each trial potentially isolating a single parameter and evaluating its significance. Another pathway is to model the response of a population of cells on the basis of their known biological response to radiation and cytotoxins, particularly at different stages of the cell cycle.

Tumour growth models have been previously developed based on either analytical methods[2, 3, 4] or probabilistic functions[5]. In the analytical approach the model is specified as a set of equations (mainly differential), and the equations are solved for an exact solution. However, analytical techniques in general do not have the flexibility to enable the variation of different parameters (e.g. cell movement, tumour proliferation) which can be incorporated with probabilistic modelling. One of the earliest models for tumour growth and cell cycle simulation using the Monte Carlo approach is CELLSIM[5]. CELLSIM operates with a large initial number of cells, placed in different phases of the cell cycle. Cells are not followed individually because of the significant number of parameters, but are modelled in groups. Therefore each group enters and exits a state together. When the number of groups reaches a certain limit, a reassignment algorithm will combine them making larger groups, where the new parameters are calculated using the weighted average of the previous ones.

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Received: 1 August, 2002; Accepted: 20 November, 2002

In the present paper, a computer generated virtual population of characteristic head/neck tumour cells has been developed using a stochastic method of cellular generation starting from an initial single stem cell. The model is an extension of the previous works as it follows each individual cell from birth to death. Tumour composition and development can be assessed in time, as well as cell age distribution. Through the use of Monte Carlo techniques and probability functions, the continued division of a cell and its daughters can be followed up to the point of detectability (10^9 cells) or lethality (10^{12} cells). The probability functions were based on established cellular behaviour, as described in the following sections, and then refined for conformity with macroscopic tumour patterns.

The establishment of a workable model will provide a virtual but realistically characteristic population of tumour cells with which the interaction and cooperation of radiation and chemotherapy can be examined. This has the potential to provide a mechanism-based combination of radio/chemotherapy in the future. At this time, the model focuses specifically on head and neck tumours due to the lack of a collation of reliable data in the literature on the broad spectrum of tumour growth and constitution parameters.

Biological foundation for model development

Normal cells as well as tumour cells propagate through a sequence of four phases (Figure 1) constituting the cell cycle: mitosis (M) when the cell divides in two daughter cells, the postmitotic phase or the first "gap" (G_1) during which the cell prepares for DNA synthesis, the synthetic phase (S) when the DNA is duplicated and the postsynthetic phase or the second gap (G_2) when the cell prepares for division[6]. Some cellular types, after mitosis, enter a resting phase called G_0. This phase is out of the cell cycle, and the resting (quiescent) cells may remain in G_0 indefinitely or re-enter the cell cycle in response to an external stimulus.

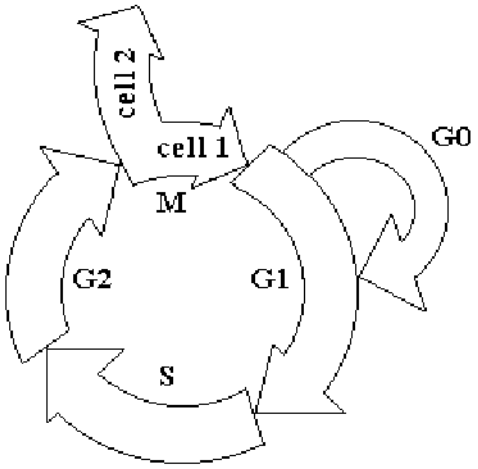

Figure 1. *Schematic representation of the cell cycle.*

The duration of a cell cycle varies from one cell generation to the next, leading to a cell population that is distributed exponentially around the cell cycle[7] at any one point in time. The variation of the cycle time between individual cells[8] is a truncated gaussian distribution with a mean value for head and neck cancer of 33 hours and a standard deviation of 13.7 hours. Truncation of the function limits the range to biologically functional values between 20 and 60 hours.

The lengths of the various phases of the cycle have been determined by cytometric measurements[9]. The only phase keeping consistency for different cell lines is the S phase, and represents one third of the whole cell cycle time[9] while mitosis is the shortest (1-2 hours) and G_1 is the most variable, but usually the longest.

There are three basic cellular types: stem (S cell), proliferative (P cell) and non-proliferative (N). In head and neck tumours, there are less than 2% S cells and up to 85% N cells[10] i.e. the S:P:N ratio is 2:13:85. Stem cells are considered to be able to indefinitely proliferate[11] while a proliferating cell is restricted to a finite number of cell divisions. A finitely proliferating cell undergoing mitosis creates another proliferative cell and, as a second daughter, either a proliferative or a nonproliferative cell (P:N ratio). A non-proliferative cell (N cell) cannot divide; after leaving the mitotic phase (M phase) of the cell cycle, the N cell enters the resting (quiescent) phase (G_0).

Tumour growth may be classified as having two distinct periods: the latency period and the clinical-growth period. The latency period starts with the initial mutation of the normal cell and lasts until the tumour grows into a clinically detectable size (10^8-10^9 cells representing ~1g of tumour mass)[10]. The clinical-growth period is of the order of one third of the latency period; the tumour requires about 30 doublings in volume to grow from a single cell to a detectable mass but just a further 10 doublings is required to achieve a lethal size (1kg, 10^{11}-10^{12} cells)[10]. For head and neck cancers, the mean tumour doubling time is 45 days[8] therefore, after 40 doublings, a single mutated cell develops into a detectable tumour in approximately 5 years. The overall latency period for a head and neck tumour is much longer, but in the present work the very initial stage of the various mutations of a normal cell into a mutated one is not considered. Because of computational-memory limitation the tumour developed by the computer grows to a microscopic size so extrapolation is used to obtain the clinically detectable-sized tumour.

In contrast to tumour growth, limitations in physical space, delivery of nutrients and oxygen, apoptosis and necrosis all lead to a substantial cell loss within the tumour. For head and neck cancer, this cell loss is as high as 85%[11].

Methods

Model description

The growth of a tumour has been modelled using probabilistic functions sampled by computer generated random number sequences i.e. the Monte Carlo method.

The model maintained the biological constitution of a tumour through the generation of stem, finitely proliferating and non-proliferating cells. Non-cancerous cells and necrotic (dead) cells within the tumour were not taken into consideration by this model as they did not contribute to the targeted goal of a virtually grown tumour having all the characteristics described above.

The modelling process was comprised of four main stages: set up, cell generation and characterisation, timing control, and result calculation and display (see flow chart of Figure 2).

The set up module defined and initialised program variables: the overall number of cells to be tracked, the S:P:N ratio, the stage of the cycle (the relative lengths of the phases of the cell cycle), the average cell cycle time, the cell loss factor, the number of generations of proliferative cells and the P:N ratio.

Starting from a single stem cell, the cell generation module initiated the creation of new cells, being the software equivalent of the biological stage of mitosis. Three pathways could be followed within the module depending on the input cell being either a stem, a finitely proliferating or a nonproliferating cell. A stem cell divides in two daughter cells, one of them being another stem in accordance with the self-renewal property of stem cells. The cellular type of the second daughter cell was sampled randomly from a uniform distribution in proportion with the biological S:P:N ratio. A proliferative (P) cell that underwent mitosis resulted in a proliferative cell with a decremented number of proliferations and a second cell with the type randomly selected from a uniform distribution in proportion with the required P:N ratio. A 50:50 ratio for P:N was considered initially but a final value of 30:70 was determined on iterative refinement of all the cell parameters to achieve agreement with accepted characteristics.

Each newly created cell was assigned a cell cycle time by randomly sampling from a truncated gaussian distribution with a mean value and standard deviation that reflected known biological characteristics. Similarly, the duration of the four phases for each cell were attributed in accordance with the following proportions of the cell cycle: M-7%, G_1-40%, S-30% and G_2-23%. An 85% cell loss of non-proliferative cells was incorporated through sampling from a uniform distribution immediate on cell generation as well as every third generation of finitely proliferating cells.

The control of the flow of cell creation and promulgation was temporally based. The first stem cell was defined as entering mitosis at time zero. Each cell created thereafter was attributed a start time and an end time. The start time was the sum of the duration of all its preceding generation's cell cycle times since time zero. The end time was the start time plus the cell cycle time of the current cell. At each interval of a master clock, each cell was scanned to see if its end time fell with the clock interval and was processed accordingly.

The results and display module kept track of the overall number of cells, the number of particular cell types and also cell distribution along the four phases of the cycle. These parameters were listed every 100 hours of biological growth time. A growth rate factor (GRF) was also determined as the ratio of cell counts between two consecutive 100 hour intervals.

Model sensitivity study

The constitution and response of the cell population at any one time has, at the current level of knowledge, an unspecified relationship with the cell characterising parameters and probability distribution functions. Simple redefinition of one of the parameters (e.g. the proportionality of S:P:N) at the start of the growth simulation will not necessarily generate the required biological constitution and growth factors, because the other parameters (cell cycle time, cell loss factor, P:N ratio) may influence the outcome. To provide an initial evaluation of these interactive processes, each parameter was individually iterated to establish its impact on the tumour's development and response.

The parameters and their incremental ranges (within realistic values as per published data) used in this sensitivity study were as follows :

1. probability of S cell creation (1.5% – 12%),
2. probability of P cell creation (1% - 25%),
3. the P:N ratio (10:90 – 50:50),
4. the mean cell cycle time (20h – 60h),
5. N cell loss (10% - 99%) and
6. number of generations of P cells (1 generation – 5 generations).

Results and discussion

Cell population development and characteristics

An initial set of parameters leading to the required macroscopic tumour behaviour was established through manual iteration of the above described probability factors. The respective ratios were: a stem cell creation probability of 1.9%, a P cell creation probability of 6.1%, a P:N ratio of 30:70, a mean cell cycle time of 33 hours, a N cell loss factor of 85% and a P cell lifetime of three generations. The growth in the number of cells of the virtual tumour under constant conditions (cell loss only from natural causes) was exponential as required[12] (Figure 3). The mean volume doubling time was 50 days, which is comparable with the biological median of 45 days (and within the range of 33-150 days)[11].

The tumour growth rate starting with different seeds for the random number generator is presented as a function of time in Figure 4. The initial variances at the microscopic level of tumour growth were due to statistical fluctuations. Tumour progression, even among tumours of the same histopathological type, can vary widely as a function of their intra- and extratumoral environment[12]. Therefore the initial fluctuations in tumour growth illustrated by the model are analogous with the growth pattern of biological tumours. The convergence towards a stable growth rate factor is characteristic for the tumour model and again is consistent with the behaviour of a biological tumour.

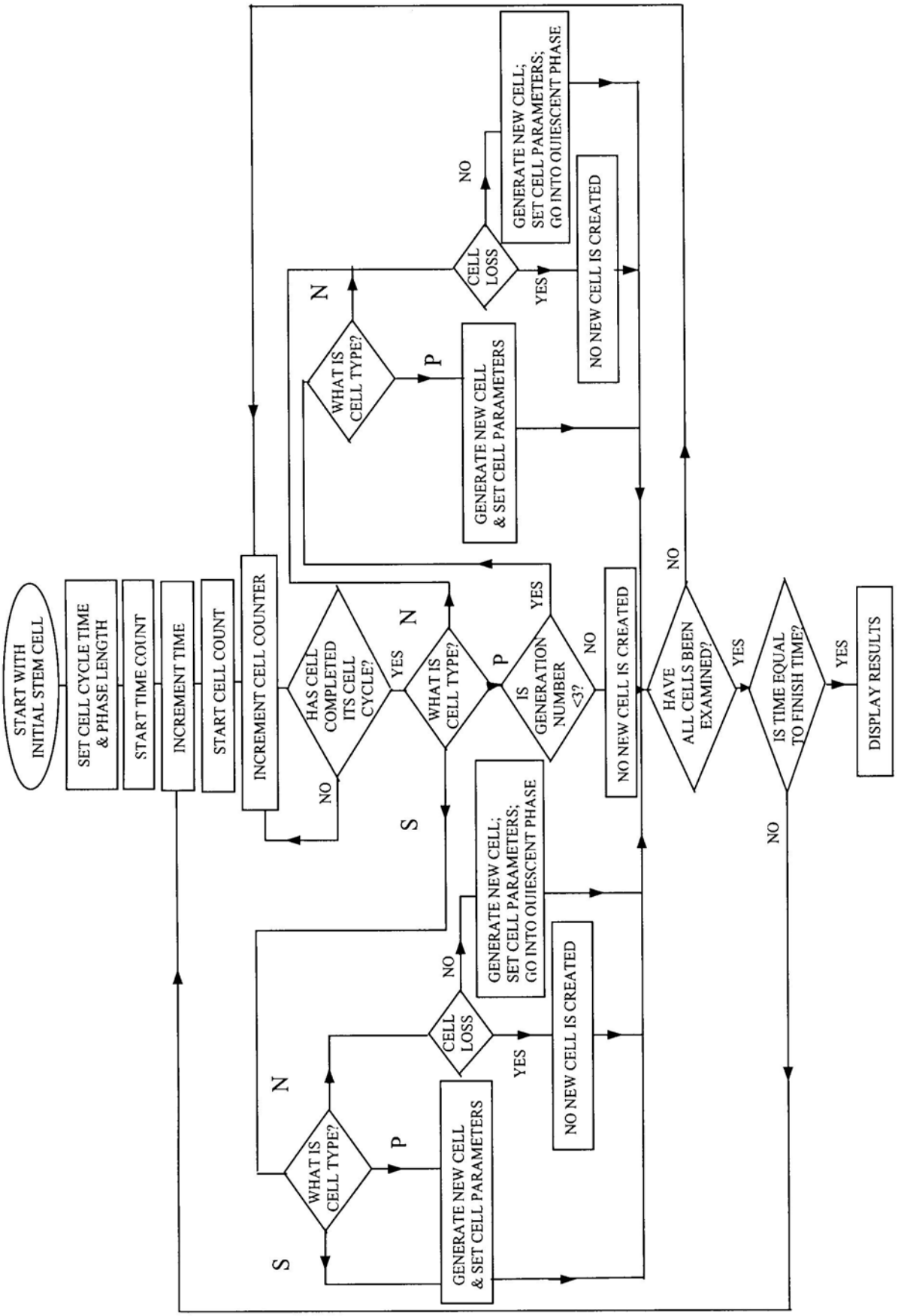

Figure 2. *Tumour growth model : flow chart.*

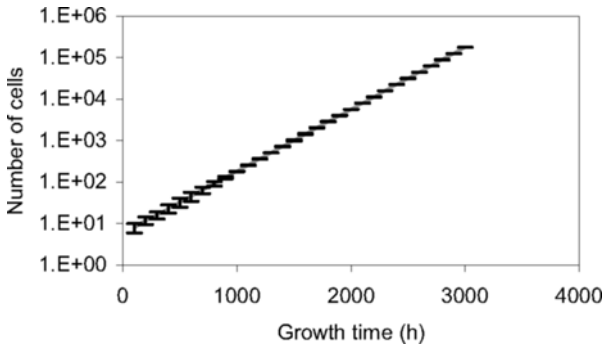

Figure 3. *The exponential growth of an untreated tumour (semi-logarithmic scale).*

The ratio between the latency period and the clinical-growth period has been calculated for the modelled tumour at 3:1 which also was in accordance with the literature[8]. This ratio is independent of initial cell probabilities (S, P or N), overall number of cells and growth rate factor and does not depend on the initial state (seed) of the random number generator.

The initial set of manually derived best-fit probability parameters to the macroscopic tumour behaviour also provided a tumour cell population of the required biological composition. An exponential distribution of cells (Figure 5) and the proportionality between the populations of the four phases was maintained as the tumour grew. However, this manual iterative process does not necessarily provide a unique or optimum result.

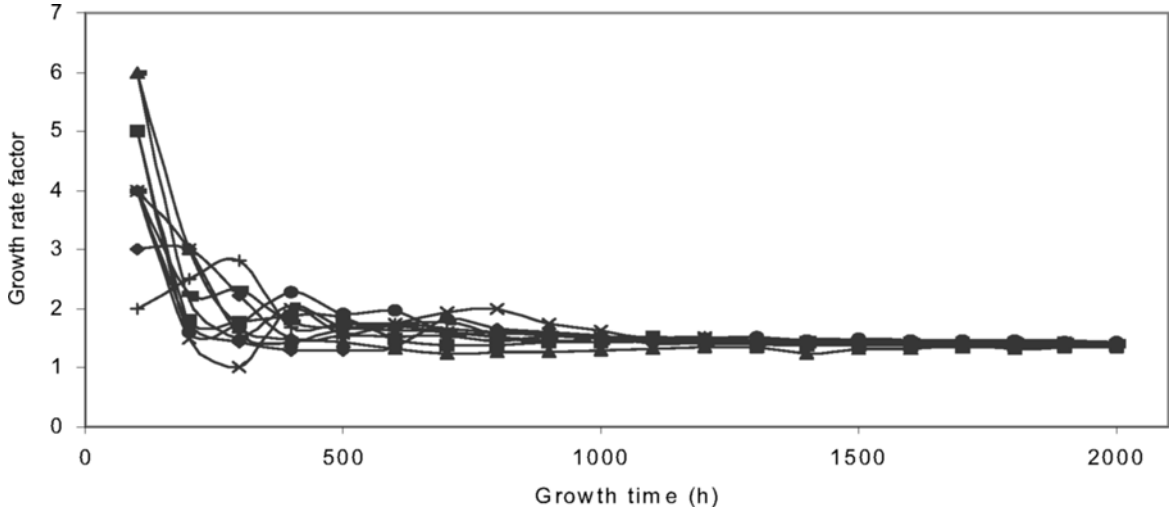

Figure 4. *Growth rate factor as a function of tumour growth time.*

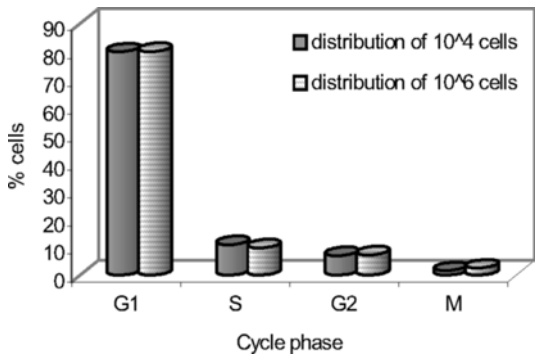

Figure 5. *Cell distribution along the cell cycle for 10^4 cells and 10^6 cells respectively.*

Sensitivity of model to probability distributions

The growth rate factor as a function of stem cell creation percentage, plotted on a linear scale, and also the tumour growth for different stem cell percentages on a semi logarithmic scale are shown in Figure 6. The error bars on the "growth rate factor" curves represent the standard deviations from a median growth rate factor for different starting seeds of the random number generator.

For low values of stem cell creation probability (1.5%-2%), the slope of the growth rate factor curve is close to zero, however the number of tumour cells increases exponentially. With greater probability values (2%-12%) the growth rate factor increases significantly and similar increase in slope is observable for the cell-number curve. This change in growth rate factor (that also led to a steeper tumour-growth curve) is due to the properties of stem cells. By increasing the initial percentage of stem cells more viable cells are created and less cells are lost (no cell loss from S cells).

The growth rate factor curve (Figure 7) was not influenced when different probabilities were set for the creation of P cells. This outcome reflects that the P cell creation is being matched by the P cell loss after the prescribed number of generation cycles. Likewise, only a slight increase in tumour growth was achieved with an increased P cell creation probability.

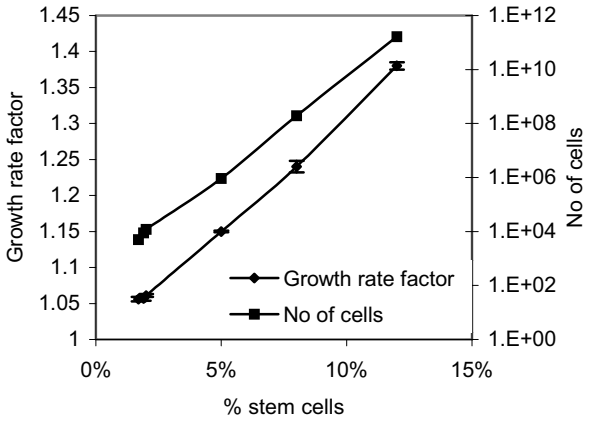

Figure 6. *Growth rate factor and number of cells as a function of the probability of stem cell generation.*

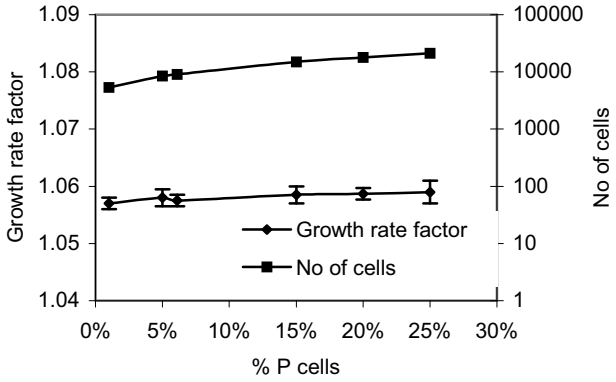

Figure 7. *Growth rate factor and number of cells as a function of the proliferative cell generation probability.*

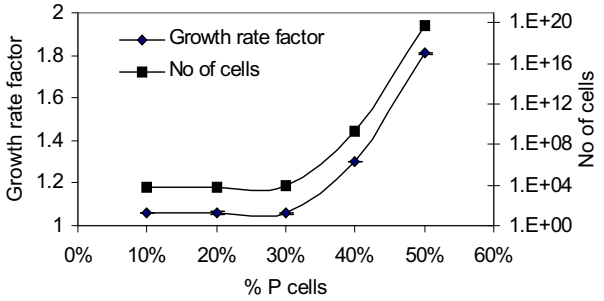

Figure 8. *Growth rate factor and number of cells as a function of the P:N ratio (the abscissa is in terms of the numerator of the P:N ratio).*

By comparing Figures 6 and 7, the major difference between stem and finitely proliferating cells can be deduced. Their different capacities for proliferation greatly influence the growth rate factor of a tumour and hence explain why the main targets in cancer treatment are the stem cells, as they are able to regenerate the whole tumour. The value of 6.1% as an initial set up for proliferative cells was chosen to achieve the biological S:P:N ratio, and to control the growth of the tumour in achieving the 50 days volume doubling time.

Figure 8 illustrates the influence of P:N ratios on growth rate factor and also on tumour growth. The optimal

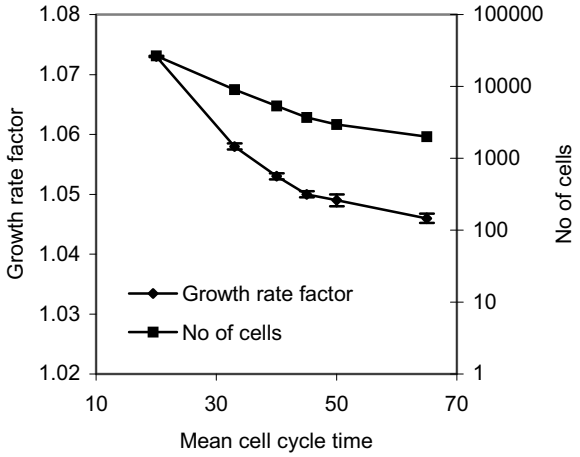

Figure 9. *Growth rate factor and number of cells as a function of the mean cell cycle time.*

P:N ratio for the model was determined by iterative processes, contributing, in the same way, to the tumour growth control as the S:P:N ratio.

While a P cell proliferates for a finite number of generations, an N cell rests in the quiescent state, G_0, out of the cycle, not capable of division. Furthermore, the cell loss due to N cells is more significant (85%) than the cell loss caused by the P cells (every 3rd generation). Therefore, the greater the P:N ratio, the steeper the tumour growth curve and more pronounced the growth rate factor. Up to the 30:70 ratio the growth rate factor is nearly constant and the tumour growth slow. There is an increased growth rate factor and number of cells at the 35:65 ratio and these keep increasing with higher P:N ratios.

The growth rate factor and the tumour growth for different mean cell cycle times is shown in Figure 9. For longer cell cycles tumour proliferation is slow as less cells enter mitosis, while for short cycle-times the tumour grows more rapidly.

For the untreated tumour, cell loss occurs mainly because of lack of nutrients associated with limitation in blood supply. The impact of the number of generations (allowed mitosis) of P cells is presented in Figure 10.

With small numbers of generations (1-3), there is a slight increase in tumour growth, while for cell loss in more advanced generations (greater than 3) the growth curve becomes steeper and the growth rate factor curve as well. The longer the generation chain, the greater the P cell population with less the cell loss, thus the larger the slope of the growth curve.

An increase in the percentage of N cell loss does not influence the growth of the tumour to the same extent as that of P cell loss. The growth rate factor decreases slightly with the cell loss, the same change being observable for the tumour growth curve (Figure 11).

In summary, the probability of stem cell creation needs to be small, the probability of proliferative cell creation has to be small enough to keep the biological proportion between stem and nonproliferative cells but sufficiently large to contribute to the tumour growth. The P:N ratio

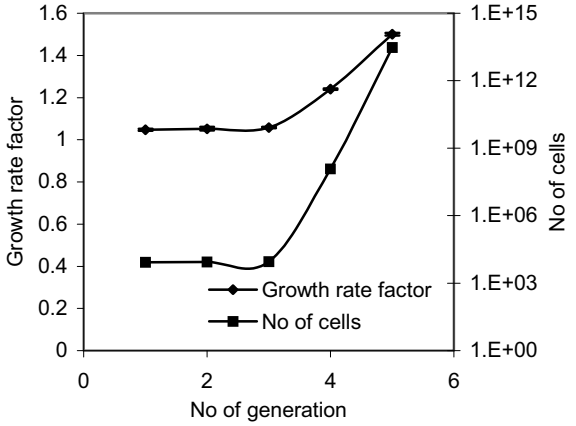

Figure 10. *Growth rate factor and number of cells as a function of the number of generations attributed to P cells.*

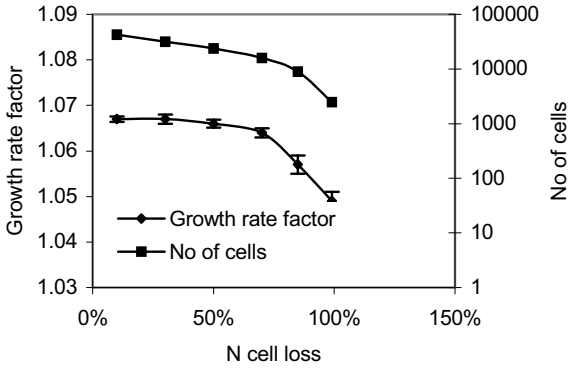

Figure 11. *Growth rate factor and number of cells as a function of cell loss from N cells.*

needs to equilibrate the production of proliferative : nonproliferative cells in order to control tumour growth. Cell loss due to both proliferative and nonproliferative cells further contributes to maintain tumour characteristics within biological parameters.

Conclusion

The biological growth of a tumour has been modelled through the application of probabilistic functions and cellular characteristics to a Monte Carlo methodology. The resultant cell population was compared with accepted biological tumour constitution and growth characteristics and agreement was achieved in terms of the exponential distribution of tumour growth, the volume doubling time and an exponential distribution of cells along the cell cycle.

The development of a virtual population of tumour cells offers a mechanism for further study and understanding of the impact of different factors on the tumour growth, potentially highlighting the situations where treatment is most effective. Further study of the model is required to evaluate if there is an optimal value for each of the proportionality factors examined as well as other factors contributing to cell growth such as hypoxia and angiogenesis. A valid model then has the potential to provide a basis for investigations into tumour repopulation mechanisms and their contribution to the 'kick-off' time studied as well as the external stimuli provided by radio and chemotherapy to the quiescent cell population.

References

1. Vikram B., Strong E.W., Shah J.P., et al. *Failure at distant sites following multimodality treatment for advanced head and neck cancer*, Head Neck Surg. 6:730-733, 1984
2. Swan, G.W., *Role of optimal control theory in cancer chemotherapy*, Math Biosc. 101:237-284, 1990.
3. Aroesty, J., Lincoln, T., Shapiro, N., Boccia, G., *Tumour growth and chemotherapy: mathematical methods, computer simulations, and experimental foundations*, Math Biosc. 17:243-300, 1973.
4. Wheldon, T.E., *Mathematical models in cancer research*, Adam Hilger Publ., Philadelphia, 1988.
5. Donaghey, C.E., *Cell cycle simulation made easy*, Int Rew Cytol. 66:171-211, 1980.
6. See any "Cell biology" text book
7. Hall, E.J., *Radiobiology for the radiologist*, 5th ed., Lippincott Williams & Wilkins, 377-396, 2000.
8. Tannock, I.F., Hill, R.P., *The basic science of oncology*, 2nd ed., McGraw-Hill, 155-176, 1992.
9. Hall, E.J., *Radiobiology for the radiologist*, 5th ed., Lippincott Williams & Wilkins, 382-386, 2000.
10. Tannock, I.F., Hill, R.P., *The basic science of oncology*, 3rd ed., McGraw-Hill, 135-165, 1998.
11. Begg, A.C., *Cell proliferation in tumours*. In: Steel, G.G. editor, Basic clinical radiobiology, 2nd ed., Oxford University Press, New York, 14-23, 1997.
12. Steel, G.G., *The growth rate of tumours*. In: Steel, G.G. editor, Basic clinical radiobiology, 2nd ed., Oxford University Press, New York, 8-13, 1997.