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**Models of radiation treatment responses after
continuous and fractionated irradiation
with/without mild hyperthermia**

by

Bilal H. Shahine

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 of Physics
Department of Physics
Carleton University
Ottawa, Canada
July 27, 1995

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**Models of radiation treatment responses after
continuous and fractionated irradiation with/without
mild hyperthermia**

submitted by Bilal H. Shahine, B.Sc.
in partial fulfilment of the requirements for the
degree of Master of Science

Thesis Supervisor

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September 5, 1995

ABSTRACT

The treatment of patients with malignant brain tumours has been less than ideal. The best treatment modality applied currently consists of interstitial implant brachytherapy with the use of continuous low dose rate irradiation. Several studies have shown that normal tissues have a lower rate of repair than tumours which opens the possibility for optimizing the current modality and replacing it with a pulsed high dose rate irradiation treatment.

This thesis investigates the effect of employing pulsed high dose-rate treatment and applying theoretical models to quantify the cell repair kinetics after such modality. Furthermore, mild hyperthermia is applied in conjunction with the latter modality. A large degree of sensitization is observed. Moreover, a repair kinetics model under hyperthermia is derived. This models helps to account for fractionated irradiation treatment since it can be incorporated in the "incomplete repair" model. Finally, an optimum modality is achieved comprised of 1 to 2 Gy dose per fraction schedules combined with mild hyperthermia at 41°C.

ACKNOWLEDGMENTS

In the name of God ...

This work is the result of a spiritual guidance and combined efforts. I would like to express my appreciation to my supervisor Dr. Peter Raaphorst, for his advice, support and kindness. I would also like to thank my co-supervisor Dr. Cheng NG. His help and encouragement have been unwavering.

I am grateful to Drs. Bog Jarosz and Robert Clarke for reviewing a part of this thesis. Their comments and suggestions are greatly acknowledged. Also I would like to thank my colleagues at the Ottawa Regional Cancer Centre, especially, Dr. Farshad Shirazi for his kind assistance and advice. My gratitude is to Dr. David Wilkins for the lab training, after all he taught me how to do cell culture.

Finally, I thank my family for their patience. My deepest appreciation are to those to whom this thesis is dedicated; to my parents for their advice, support and encouragement throughout my life.

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*No human investigation can be called true science
without passing through mathematical tests.*

Leonardo da Vinci (1452-1519)
(in Treatise on Painting, Chap.1)

1.0 INTRODUCTION

1.1 BACKGROUND

The results of treatment of the malignant glioblastoma multiforme (GBM) (one form of brain tumour) have been less than ideal. The best reported results are generally a median survival of approximately 10 to 12 months. Many approaches have been taken to improve these results including various chemotherapeutic agents, hyperfractionation, ultra-high dose-rate radiotherapy, and interstitial implants. Only the last has appeared to impact on survival of patients with this fatal disease¹.

In interstitial implant brachytherapy (or "short distance" therapy), radioactive sources may be inserted into the body in direct contact with the malignant tissue so that a very high radiation dose can be delivered just where it is needed². A conventional brachytherapy treatment protocol uses low dose-rate (0.4 to 2 Gy/hr) irradiation. The limiting factor in the latter setting is the normal tissue tolerance which should be fully used to maximize the possibility of tumour control. However, the maximum dose that can be delivered with acceptable damage to surrounding normal tissue depends not only on the dose-rate, but also on the difference in the rate of repair of sublethal damage between malignant and normal tissues^{3,4}.

Sublethal damage (SLD) is one form of damage that, given

time, energy and nutrients, the cells can repair and recover completely from it. If more damage is accumulated before the first sublethal damage is repaired, the two may interact to become lethal¹¹. Sublethal damage occurs during low dose-rate irradiation and between fractions of a pulsed (fractionated) treatment.

Another form of damage is the potentially lethal damage (PLD) which occurs in cells after exposure to a radiation dose. It can be lethal depending on the postirradiation environment. For instance, *in vitro* (experimental cell culture) studies have shown that the addition of full growth medium after a dose of radiation inhibits the repair of PLD and transforms it into lethal damage which leads to cell death¹¹.

Therefore, in order to produce a larger response in the tumour than in the surrounding normal tissue, one has to design protocols that take into account the rates of repair of sublethal damage of both types of tissues. There is evidence suggesting that sublethal damage repair rates are slower for normal tissues⁴. If this difference is real, it opens up new possibilities for optimizing brachytherapy schedules.

In a recent study, computer optimized protocols were suggested based on maximizing the therapeutic difference between tumour-control probability (TCP), and normal-tissue complication probability (NTCP). Using a repair rate for tumour that is faster than for normal tissues, a series of

acute doses separated by 3 to 4 h produced an NTCP=0.11 and TCP=0.83 compared to NTCP=0.2 and TCP=0.8 for continuous low dose-rate regimen with the same overall time and dose. Furthermore, giving large doses per fraction (around 4 Gy) also improves the therapeutic ratio⁴.

Given this information, continuous low dose rate, as presently used in interstitial brachytherapy, does not represent the optimal schedule to produce the maximum therapeutic ratio between tumour and normal tissue. Pulsed brachytherapy or conventional external radiotherapy, in which pulses of high dose-rate (>12 Gy/h) are delivered at intervals of several hours, would result in a bigger differential between the two types of tissues⁵.

Hyperthermia or the application of heat treatment above normal (37°C) temperature is a modality which on its own probably has a limited role to play in the curative treatment of tumours. Its main application is in combination with other cancer treatments, especially radiation⁶. In the case of glioblastoma multiforme tumours, even after interstitial implants, local recurrence continues to be the most common mode of failure⁷.

However, parallel to the efforts toward improving brachytherapy protocols, researchers have had great interest in hyperthermia as an adjunct to interstitial radiotherapy because of the potential synergistic effect of the two modalities in achieving local tumour control⁷. This effect

is termed as heat/thermal radiosensitization. Several studies have shown that hyperthermia inhibits the repair of radiation induced strand breaks of DNA, especially at temperatures above 42°C. Little is known about the mechanisms of hyperthermia below 42°C^{8, 67}.

Hyperthermia has been shown to be more effective in thermal radiosensitization in cells that have a large capacity for recovery. As expected, previous studies have shown that human glioma cells are resistant to radiation due to their ability to repair damage. Moreover, studies on normal glial cells showed no indication of a radiation survival curve shoulder and may thus have reduced repair capacity. Thus hyperthermia in the treatment of brain tumours may cause a greater effect on tumour cells than normal cells⁹. Therefore, a pulsed HDR irradiation combined with hyperthermia treatment may be the optimal modality in treating patients with glioblastoma multiforme.

1.2 THESIS OBJECTIVES AND OUTLINE

The scope of this research is to investigate the response of human glioma cells to pulsed (fractionated) HDR irradiation, including the addition of hyperthermia treatment. In our approach we will use theoretical models in interpreting and explaining experimental results. However, models do not exist especially in the combined treatment of radiation and hyperthermia. Therefore, our plan of action will consist of

three major steps:

- 1) Investigate the effect of continuous HDR treatment, apply various models to the data and evaluate their goodness-of-fit. This step is important since most models that describe the response of fractionated irradiation are generalizations of the HDR continuous irradiation treatment models. Further, we will quantify the radiosensitivity parameters of this cell line from these various models.

- 2) Apply pulsed HDRI treatment modalities and compare them to two continuous low dose rate treatments. In both cases of fractionated HDRI and of continuous LDRI there is a chance for cells to repair sublethal damage. A model that describes the last two modalities will be fitted and repair kinetic coefficients will be estimated.

- 3) Combine hyperthermia with the pulsed HDR modalities and assess the degree of radiosensitization resulting from this treatment. There is no model that accounts for this combination. Therefore, a model will be derived and data analysis will be presented.

This thesis comprises five chapters and an Appendix. The first chapter is an introduction and the last one is a conclusion and a statement of areas for future research. The three above objectives are discussed in three separate chapters. The Appendix deals with statistical methods and analysis used in the second chapter.

2.0 CONTINUOUS IRRADIATION TREATMENT: FITNESS AND COMPARISON OF DIFFERENT MODELS

2.1 INTRODUCTION:

Among the developments stimulated by experimental research are theoretical approaches to the quantisation of the radiobiological phenomena produced. A motivation is to arrive at a unified understanding of the underlying physical and biological mechanisms. In an entity as complex as a living cell, it is not unreasonable to expect that there may be several, perhaps many, levels of models to be developed which will ultimately provide the quantitative expression of our understanding of the processes leading to a particular end point¹⁰.

Qualitatively, the shape of the survival curve can be described in relatively simple terms. At "low doses" for sparsely ionizing radiations, such as x-rays, the survival curve (from zero to 2 or 3 Gy dose range) starts out straight on the log-linear plot with a finite initial slope; that is, surviving fraction is an exponential function of dose. At higher doses the curve bends inward. This bending or curving region (from 3 to 6 Gy) extends over a dose range of a few Grays. At very high doses (greater than 8 Gy) the survival curve often tends to straighten again; the surviving fraction returns to being an exponential function of dose¹¹.

The literature contains a number of models for the effects of radiation on cell survival. Although there is no widespread agreement on which of the numerous models of cell survival is best, it is widely accepted that a two-component model is necessary to adequately describe cell survival *in vitro*¹².

Basically, fitting models to data is multipurpose: first, the hypothesis underlying the models can be tested and may give insights into the mechanisms of radiation action. In addition, comparison of fitting accuracy of different models gives the opportunity to reject some of them, and hence their underlying mechanisms. Second, in order to measure and compare radiation sensitivity for different cell lines, radiobiologists have quantified the changes in dose response curves as a function of independent parameters derived from models.

Three survival-curve models, chosen on the basis of their totally differing hypotheses, will be summarised and compared for the case of high dose-rate continuous irradiation treatment (HDRI).

1. The linear Quadratic model

This theory was presented by Chadwick and Leenhouts¹³ to explain the effect of radiation on cell survival on the basis of the assumption that a double strand break in the DNA helix is the critical damage. The foundations of their theory at the

molecular level can be summarised as follows:

a) The reproduction of cells is governed by the activity of certain critical molecules which are the DNA.

b) A critical damage is to be a double strand break in the DNA.

c) The primary action of radiation on the cell is considered to cause molecular bond breaks in the DNA strands. Any modification of this primary damage is considered to be a repairing or misrepairing action.

d) The various radiobiological effects found in cell type under different irradiation conditions reflect varying degrees of repair.

e) These repair processes are considered to embrace the physical recombination processes and energy transfer, the chemical restitution processes and the biochemical enzymatic repair processes.

Analytically, the production of DNA single strand breaks (ssb) and double strand breaks (dsb) by ionizing radiation is presented as follows:

** For DNA single strand breaks:*

Let N_0 be the number of initial critical bonds per unit mass which will lead to a single break if damaged. Let K be the probability per bond per unit dose that the bond is broken, and let D be the dose, then

$$\frac{dN}{dD} = -KN \quad (1)$$

where N is the number of unbroken bonds per unit mass, and hence

$$N = N_0 \exp(-KD) \quad (2)$$

so that the number of broken bonds per unit mass is

$$N_0 - N = N_0 (1 - \exp(-KD)) . \quad (3)$$

If r is the fraction of repaired broken bonds, then the fraction of unrepaired broken bonds is $f = 1 - r$, and the mean number of DNA ssb is

$$fN_0(1 - \exp(-KD)) \quad (4)$$

* for DNA double strand breaks:

The DNA double helix may be broken in two modes of radiation action:

- i) both strands are broken in one radiation event, or
- ii) each strand is broken independently in different radiation events.

In dealing with the second mode let:

n_1 = number of critical bonds per cell on strand 1,

n_2 = number of critical bonds per cell on strand 2, then

$$n_1 = n_2, \quad (5)$$

Let K be the probability per bond per unit dose that the bond is broken, and f_1 and f_2 be the proportion of broken bonds on each unrepaired strand, then if a proportion Δ of the dose D inactivates via the first mode of action and $(1 - \Delta)$ is the

proportion giving the second mode of action, then the number of unrepaired broken bonds on strand 1 is

$$f_1 n_1 [1 - \exp[-kD(1-\Delta)]] \quad (6)$$

and on strand 2 is

$$f_2 n_2 [1 - \exp[-kD(1-\Delta)]] \quad (7)$$

If ϵ is the proportion of these broken bonds which combine to produce a dsb and are available to cause cell death, the mean number of dsb per cell is

$$\epsilon n_1 n_2 f_1 f_2 [1 - \exp[-kD(1-\Delta)]]^2 \quad (8)$$

For the first mode of action (i), let n_o be its number of sites where $n_o \leq n_1, n_2$, and let k_o be the probability per site per unit dose that the double strand is broken, then the mean number of DNA dsb formed by this mode of action is

$$n_o [1 - \exp(-k_o \Delta D)] \quad (9)$$

Thus the mean number of DNA double strand breaks per cell after a dose D is given as:

$$n_o [1 - \exp(-k_o \Delta D)] + \epsilon n_1 n_2 f_1 f_2 [1 - \exp[-kD(1-\Delta)]]^2 \quad (10)$$

If f_o is the proportion of DNA double strand breaks not repaired and assuming that double strand breaks are randomly distributed in DNA, then using Poisson statistics the probability of finding j double strand breaks is

$$P(j) = \frac{(dsb)^j}{j!} \exp(-dsb) \quad (11)$$

where dsb is just the number in equation(10) multiplied by f_3 .

A cell will survive if it contains no critical damage, thus

$$S = P(0) = \exp(-dsb) \quad (12)$$

and, therefore

$$S = \exp(-pf_o(n_o[1-\exp(-k_o\Delta D)] + \epsilon n_1 n_2 f_1 f_2 [1-\exp[-kD(1-\Delta)]]^2) \quad (13)$$

where p is a proportionality factor connecting DNA dsb and cell death.

As K_o and K are very small, the survival after approximating the two inner exponentials to the first order term of a Taylor series will be

$$S = \exp[-pf_o(n_o k_o \Delta D + \epsilon n_1 n_2 f_1 f_2 [k(1-\Delta)D]^2)] \quad (14)$$

For simplicity, the following notation is adopted:

$$\alpha = pf_o n_o k_o \Delta \quad (15)$$

$$\beta = pf_o \epsilon n_1 n_2 f_1 f_2 [k(1-\Delta)]^2 \quad (16)$$

Finally, the survival probability will be given by

$$S = \exp(-\alpha D - \beta D^2) \quad (17)$$

The above formula is general for all ionizing radiation and contains a factor (Δ) which depends on the Linear Energy

Transfer (LET) of the radiation. Therefore, α and β will take different values respectively depending on the LET of the radiation used. At very low doses the linear term in the dose dominates, whereas at higher doses the survival will be dominated by the quadratic term.

2. Lethal-Potentially Lethal Model

In the history of radiobiological models there were various attempts to describe the processes involved in the expression of damage as a sequence of intermediate stages. S.B. Curtis¹⁰ produced a unified model for cellular radiobiology by merging certain features of the cybernetic model¹⁴ and the Repair-Misrepair (RMR) model¹⁵. The model, presented below, is based on the following hypotheses:

(i) Two different kinds of biological lesions relevant to cell killing are created in a cell: "lethal" and "potentially lethal" lesions. Potentially lethal lesions can be repaired or may also interact with each other to produce lethal (irreparable) lesions. This process depends on the square of the lesion concentration and can be called a "misrepair" process. The probabilities of correct repair and misrepair depend on the "initial proximity" of one lesion to another and their overall concentration.

(ii) Cell survival is derived on the basis of a Poisson distribution in the number of lesions per cell after the available time of repair has elapsed.

(iii) It is assumed that the mean numbers of both kinds of lesions are formed at rates proportional to the absorbed dose rate.

(iv) The rate of repair per lesion is assumed to be independent of lesions present which means that there is no saturation in the repair process.

With the above hypotheses, the LPL model was developed and the survival curve expression is derived in the same way as the cybernetic model except for the misrepair term depending on the square of the concentration. Therefore, the time rate of change of the mean numbers of potentially lethal ($n_{PL}(t)$), and lethal ($n_L(t)$) lesions during the irradiation period using dose rate D is

$$\frac{dn_{PL}(t)}{dt} = \eta_{PL}\dot{D} - \epsilon_{PL}n_{PL}(t) - \epsilon_{2PL}n_{PL}^2(t) \quad (18)$$

$$\frac{dn_L(t)}{dt} = \eta_L\dot{D} + \epsilon_{2PL}n_{PL}^2(t). \quad (19)$$

where η_{PL} and η_L are the rates per unit absorbed dose for production of the potentially lethal and lethal lesions, respectively. The ϵ_{PL} and ϵ_{2PL} are the rates per unit time of correct repair and binary misrepair, respectively, for the potentially lethal lesions.

The initial conditions are $n_{PL}(0) = n_L(0) = 0$; i.e., no lesions are assumed to be present at the start of the irradiation. The solutions of these equations can be written

as:

$$n_{PL}(t) = \frac{2\eta_{PL}\dot{D}(1 - e^{-\epsilon_o t})}{\epsilon_o + \epsilon_{PL} + (\epsilon_o - \epsilon_{PL}) e^{-\epsilon_o t}} \quad (20)$$

where

$$\epsilon_o = (\epsilon_{PL}^2 + 4\epsilon_{2PL}\eta_{PL}\dot{D})^{1/2} \quad (21)$$

and

$$n_L = \eta_L D + \epsilon \ln \left[\frac{2\epsilon_o}{\epsilon_o + \epsilon_{PL} + (\epsilon_o - \epsilon_{PL}) e^{-\epsilon_o t}} \right] + \frac{(\epsilon_o - \epsilon_{PL})^2 t}{4\epsilon_{2PL}} - n_{PL}(t) \quad (22)$$

where

$$\epsilon = \epsilon_{PL} / \epsilon_{2PL} \quad (23)$$

Thus, the total mean number of lesions is

$$n_{TOT}(t) = n_L(t) + n_{PL}(t) \quad (24)$$

Assuming that potentially lethal lesions cause cell death if not repaired before cells are plated for survival and using the Poisson assumption, we get the survival probability as follows:

$$S = e^{-n_{TOT}(t)} \quad (25)$$

If we assume the irradiation stops at time T, we have similar equations for the repair of lesions in the postirradiation period but without the source terms involving the dose rate.

In order to calculate the survival curve expression at

time $t = T + t_r$ (where t_r is the time available for repair after the end of the exposure), it is assumed that the total mean number of lethal lesions per cell is the sum of the lethal and potentially lethal lesions. That is all potentially lethal lesions still present at the end of available repair time, t_r , are fixed. Finally,

$$S(D, \dot{D}, t_r) = e^{-N_{TOT}(D/\dot{D})} \left[1 + \frac{N_{PL}(D/\dot{D})}{\epsilon} (1 - e^{-\epsilon_{PL} t_r}) \right] \epsilon \quad (26)$$

where N_{TOT} is the sum of lethal (N_L) and potentially lethal (N_{PL}) lesions after the end of the radiation exposure and after a time t_r of repair. Again, D is the absorbed dose.

The overall shape of a survival curve obtained at high dose rates using the above formula has the following characteristics:

- (i) There is an initial nonzero slope.
- (ii) There is a region of this curve at low dose that can be approximated by a linear-quadratic function in the absorbed dose.
- (iii) At high doses, the slope of the survival curve approaches a constant.

3. Saturable Repair Model

Most quantitative models in radiobiology make the implicit assumption that all relevant repair processes proceed in a dose-independent manner. They assume that the "shoulder" portion of survival curves is primarily due to the interaction

of sublethal lesions or the accumulation of sublethal damage. By contrast, D.T. Goodhead¹⁶ in his saturable repair model assumes that the repair process of relevant damage is dose-dependent in that the rate of repair is not directly proportional to the amount of damage present after different doses of radiation. Therefore, the shoulder portion is the result of the repair of single lesions produced by small doses of radiation, and with increasing dose the repair process becomes saturated. This model is based on the following assumptions:

a) Repairable radiation lesions are produced locally within the cell by 1-track action of ionizing radiation and in direct proportion to dose D . Hence the initial number of such lesions is

$$n_0 = aD \quad (27)$$

where a is a proportionality constant depending on radiation quality.

b) Some of these lesions are repaired by a system whose efficiency decreases with increasing dose due to partial saturation of repair kinetics.

c) For a very simple form of saturable repair, the rate of repair of lesions is given by

$$\frac{dn}{dt} = -kcn \quad (28)$$

where c is the number of repair molecules or enzymes, n is the number of unrepaired lesions and k is a proportionality rate

constant.

d) For simplicity the repair molecules are assumed to be not recycled. Therefore, repair of each lesion reduces c by 1, i.e.,

$$dc = -dn \quad (29)$$

e) Repair proceeds until time T , at which time all residual lesions become "fixed" and no longer repairable.

Hence the residual number of lesions after repair can be determined by integration between 0 and T which yields

$$n_T = \frac{(n_0 - c_0)}{1 - \frac{c_0}{n_0} \exp[kT(c_0 - n_0)]} \quad (30)$$

Using equation (27), the dose-effect curve is obtained as

$$\epsilon(D) = pn_T \quad (31)$$

where p is the proportion of the lesions which are capable of producing the observable biological effect.

Again cell survival may be obtained by using Poisson statistics and hence

$$S(D, T) = \exp\left(-\frac{p(aD - c_0)}{1 - \frac{c_0}{aD} e^{[kT(c_0 - aD)]}}\right) \quad (32)$$

Some features of the above model are as follows:

(i) The dose-response curves may have a nonzero initial slope even when it is assumed that all lesions are of a

repairable type.

(ii) At high doses, the final slope (paD) is a measure of the number of initial critical lesions produced before repair.

(iii) Linear extrapolation of the high dose portion back to zero dose gives pc_0 .

(iv) The initial slope ($paD \exp(-kTc_0)$) is not a direct measure of lesion production but it includes the exponential term for the probability of a lesion produced at low dose not being repaired.

In conclusion, the Linear Quadratic model is based on a target theory using the DNA as the critical site in the cell without accounting for a model of repair. On the other hand, the other two models do not propose a mechanism of cell killing; moreover, they differ in their understanding of the cellular repair process.

4. Radiosensitivity parameters

Parameters estimated play an important role, especially, those that are derived from the Linear Quadratic (LQ) model. It is customary to report the radiosensitivity of a given cell line as the ratio of α/β derived from fitting the LQ model to survival curve data^{17,18}. This ratio defines the dose for which the two types of damage discussed earlier contribute equally to the response¹⁹.

A second measure of radiosensitivity is the surviving fraction at 2 Gy (SF2). The use of SF2 is advantageous because

it is a single parameter which may predict the probability of tumour control²⁰. Therefore, in the case of the LQ model SF2 is defined as

$$SF2 = e^{(-\alpha \cdot 2 - \beta \cdot 4)} . \quad (33)$$

A third measure is the mean of the inactivation probability density function, the so-called "mean inactivation dose", a parameter recommended for cell survival characterization by ICRU²¹. This parameter is the area under the survival curve and can be characterized by

$$\bar{D} = \int_0^{\infty} S dD . \quad (34)$$

In this chapter the results of irradiating a human glioma cell line at high dose rate are reported. This cell line was irradiated under stationary or plateau growth phase conditions. The resulting survival curve was fitted to the above three models. A goodness-of-fit test was applied in all cases and parameter estimation is presented.

2.2 MATERIALS AND METHODS

The cell line U-87MG was established from a human malignant glioblastoma²². It was originally obtained from the American Type Culture Collection cell repository catalogue. The cells were grown in the medium combination DMEM:F-12 (1:1) supplemented with 7.5% Fetal Bovine serum, 7.5% Newborn Calf serum, 1 mM MEM non-essential amino acids, 10 mM sodium bicarbonate and 20 mM Hepes. All cell cultures were incubated at 37°C in a humidified atmosphere of 2% CO₂ and 98% air.

For all experiments 4×10^3 cells per cm² were plated in 25 cm² flasks. The medium was changed (4 ml in volume) on days 4 and 7 after cell plating with experiments commencing on day ten²³.

For High dose rate irradiation (HDRI), cells were irradiated on ice with 150 KVp X-rays unit at a dose rate of 1.12 Gy/min. The dose to the cells was measured by TLD dosimetry.

Cell survival was assayed by the colony formation assay. Following treatment, the cell monolayers were rinsed with citrate saline (KCl 134 mM, citric acid 17.6 mM) to remove cellular debris and trypsin inhibiting substances. Trypsin (0.2% w/v in citrate saline) was then rinsed over the cells, and the excess aspirated. After 5 minutes at 37°C the cells were pipetted up and down until they were in a single cell suspension. The suspensions were diluted in fresh medium and counted using an electronic cell counter (Particle Data).

Appropriate cell numbers were plated in either 60 or 100 mm dishes, depending on the number of cells required to achieve 30-80 colonies per dish. Fourteen days later the cells were stained with crystal violet (0.2% w/v in 70% ethanol) and surviving colonies counting more than 50 cells were enumerated²⁴.

Typically 3 dishes were used to score the number of surviving colonies at a given treatment dose. The surviving fraction was calculated as follows:

$$S = \frac{\text{col/dish}}{\text{cells-seeded/dish}} \quad (35)$$

Based on the way the data have been collected we can distinguish three kinds of error²⁵:

(i) *Binomial* error due to radiation killing which is a random process.

(ii) *Sampling* error due to the limited number of cells pipetted in the dilution or plating procedures.

(iii) '*Gremlin*' error due to any of the inadequately controlled variables which often play a part in an experiment, for instance: temperature shock, pH, quality of serum, oxygen concentration and non-uniform mixing of the suspension.

There is no statistical technique for predicting the magnitude of any '*Gremlin*' errors.

Let N be the number of cells that have been irradiated and sampled from a uniform suspended solution. Assuming that N is known exactly, the binomial variance of the number of

cells that have survived after the radiation dose or, more specifically, of the number of colonies (c) that have formed is given by

$$\text{Var}(c) = N \cdot S(1-S), \quad (36)$$

where we have used the above definition of survival probability. Thus, the variance of the survival probability is given by

$$\text{Var}(S) = \frac{S(1-S)}{N}. \quad (37)$$

However, taking into account the sampling process, the number of cells is pipetted or sampled with an accuracy of its square-root. If N is the number sampled, then we can write

$$\text{Var}(N) = N. \quad (38)$$

In order to get the variance of the survival probability (S) that takes the sampling error into account, we use the propagation of error rule; therefore,

$$\text{Var}(S) = \left(\frac{1}{N}\right)^2 \text{Var}(c) + \left(-\frac{c}{N^2}\right)^2 \text{Var}(N). \quad (39)$$

Substituting the 2 variances in equation (39) by their equivalents from equations (36) and (38), the survival variance will be given by

$$\text{Var}(s) = \frac{S}{N} \quad (40)$$

Therefore, every survival probability at any given dose is subjected to the above variance.

Usually, the survival at a given dose does not have the same uncertainty among repeated trials. This is a direct result of equation (40), since the number of sampled cells plated for colony formation is not always chosen the same in repeated experiments. Therefore, there is a certain efficiency in taking each measurement and the average survival is formed as follows⁶⁹:

$$\langle S \rangle = \frac{\sum W_i S_i}{\sum W_i} \quad (41)$$

where

$$W_i = \frac{1}{\text{Var}(S_i)} \quad (42)$$

and its variance is given by

$$\text{Var}\langle S \rangle = \frac{[\sum W_i (S_i - \langle S \rangle)]^2}{\sum W_i} * \frac{n_{\text{eff}}}{n_{\text{eff}} - 1} \quad (43)$$

where

$$n_{\text{eff}} = \frac{(\sum W_i)^2}{\sum W_i^2} . \quad (44)$$

The zero-dose point or what is usually called the plating efficiency is treated in the same way as that for the other points in terms of error assignment. The plating efficiency usually ranges from 30 to 60% due to the failure of most of the cells to attach themselves to the bottom of the plate.

The common practice of normalizing cell survival data by dividing the raw survival by the observed plating efficiency produces biased or unreliable estimates for parameters of a given model²⁶. Therefore, we can improve the approach to normalizing cell survival curves by dividing by the true plating efficiency.

The most reliable estimate of the plating efficiency can be obtained by introducing a new parameter to the model. This new parameter takes into account not only the measured value of the plating efficiency but also the extrapolated value from the whole dose-response curve.

For instance, in the Linear Quadratic and in any model the plating efficiency parameter can be presented as an additional parameter (z for example) in front of the exponential. Thus, for the LQ model

$$S = z * \exp(-\alpha D - \beta D^2), \quad (45)$$

for the LPL model

$$S(D, \dot{D}, t_r) = z * e^{-N_{TOT}(D/\dot{D})} \left[1 + \frac{N_{PL}(D/\dot{D})}{\epsilon} (1 - e^{-\epsilon_{PL} t_r}) \right] \epsilon \quad (46)$$

and for the SR model

$$S = z * \exp\left(-\frac{p(ad - c_0)}{1 - \frac{c_0}{ad} e^{[kT(c_0 - ad)]}}\right). \quad (47)$$

Regarding radiosensitivity parameters, the calculation of the surviving fraction at 2 Gy (SF2) is based on the

parameters estimated for the different models. The mean inactivation dose is estimated using numerical integration.

2.3 RESULTS

The Growth curve for U-87MG is shown in figure 1. As we can see, the cell population grows slowly during the first few days because cells take time to adapt to the new environment and many of them fail to attach themselves to the flask. After that, the cell population starts increasing dramatically due to the abundance of nutrients and space. This phase is called the exponential phase. The third stage is the plateau phase where cell population becomes steady indicating an equilibrium between the number of newborn cells and the ones that have died. Cell density at plateau was about 10^5 cells per cm^2 .

Survival curve experiments for plateau phase cells following HDRI were repeated four times. Figure 2 shows raw survival data from one of the experiments. The LQ model is fitted to estimate its parameters and the plating efficiency using a weighted least-square fitting procedure. Shown in figure 3 is the result of fitting the four experiments to the LQ model. Every experiment (similar to figure 2) is presented by a point whose coordinates are α and β . In order to explain the variation found in the values of α and β among repeated experiments, a simulation experiment was performed (see the Appendix). Figure 4 shows an overlay of the experimental and simulated parameters of the LQ model.

Furthermore, from every single set of simulated parameters α/β , SF2 and $\bar{D}$ are derived in order to test the variation of these radiosensitivity parameters considering the sources of error of the colony formation assay. Figure 5 shows α/β vs SF2 and $\bar{D}$ for the 4 repeated experiments and figure 6 shows the same parameters derived from 1000 simulated repetitions. Figure 6 shows the correlation between simulated SF2 and $\bar{D}$ values.

Table 1 shows the LQ model estimated parameters from the four averaged experiments. The standard errors and the correlation coefficient of the two parameters α and β are those derived from the simulation experiment. The coefficients of variation, defined as the ratio of the standard error over the average value of every radiosensitivity parameter discussed above, are presented in table 2.

Figure 7 shows the weighted average of the four experiments. It has been fitted to the linear-quadratic model (LQ), the Lethal-Potentially Lethal model (LPL), the Saturable Repair model (SR) and an extension of the Linear Quadratic model which we call the **Linear Quadratic Cubic** model. This latter model is derived after fitting the LQ model when the fit showed a very small initial slope which is characterized by α .

The reason behind extending the LQ model is to achieve a better fit to survival data. On the other hand, the final form of the LQ model, equation (17), was the result of a

Taylor series expansion which is approximated to the first order term of the series. Moreover, at high doses (>10 Gy) the expansion of the first exponential to the first order may be valid; however, this is not the case for the second exponential which is squared.

Therefore, going back to equation (13), and making approximation to the second order term of the series, we will have

$$1 - e^{(-k_0 \Delta D)} = \frac{k_0 \Delta D}{1!} - \frac{(k_0 \Delta D)^2}{2!} + \dots \quad (48)$$

and for the second exponential

$$(1 - e^{[-kD(1-\Delta)]})^2 = \left(\frac{k(1-\Delta)D}{1!} - \frac{[k(1-\Delta)D]^2}{2!} + \dots \right)^2. \quad (49)$$

Neglecting terms to the fourth exponent as k is very small; therefore,

$$S = \exp(-aD - bD^2 - cD^3) \quad (50)$$

where the following relation exists between a , b , c , and α , β parameters:

$$a = \alpha, \quad (51)$$

$$b = \beta + \alpha * \frac{k_0 \Delta}{2} \quad (52)$$

$$c = \beta * k(1-\Delta). \quad (53)$$

Figure 8 shows an overlay of the four fits. An extrapolation has been made to show the behaviour of the four models at large doses such as 16 Gy. The inset shows the behaviour at small doses (<2 Gy) and indicate the initial slope predicted by each model.

The assessment of goodness-of-fit (Q) helps to compare the different models. If Q is a very small probability, then the apparent discrepancies are unlikely to be chance fluctuations. Much more probably either (i) the model is wrong (can be statistically rejected), (ii) the size of the measurement error is underestimated, or (iii) the measurement errors may not be normally distributed. Possibility (iii) is fairly common, so it is not uncommon to deem acceptable any models with , say, $Q > 0.001$ ²³.

The differences found among the four models are presented in table 3 by values of the mean inactivation dose ($\bar{D}$), and the surviving fraction at 2 Gy (SF_2). Minimum residual squares and goodness-of-fit coefficients derived from each of the models are also shown in the same table.

2.4 DISCUSSION

The data points in the survival curves have been averaged from four experiments and present the response of the cell line over a long period of time. A significant variation in the survival level has been observed among the four experiments which results in a variation in the estimated

parameters in the four models. This behaviour is seen even in different cell lines after HDRI²⁷, and may be due to a variation in radiosensitivity of the cell line over time.

In order to test this hypothesis and to get an error estimation and correlation coefficients of the model parameters, a simulation experiment was conducted. Monte Carlo simulation is the best tool to test the extent of variation of survival curve data and assess parameter fluctuations within the sources of error mentioned above²⁸. The procedure is to draw random survival curve data fluctuating around predetermined survival curve (See the Appendix).

For the sake of comparing the variation of the experimental and the simulated survival curves, the Linear Quadratic model was adopted. The choice of the LQ model was based on an earlier work²⁹ which aimed to compare and test the variation of the parameters as a function of the investigated dose regions and the resolvability of the parameters among different models. The LQ model was shown to be the most robust and its parameters have the highest resolvability compared to five other models, which include the other two models discussed here.

Therefore, each curve is represented by a point whose coordinates are α and β . The starting values of α and β were derived from averaging the survival data of the four experiments and fitting these data to the LQ model. Thus, the average of the 4 pair values of estimated α and β from the 4

experiments is not considered here. One can argue that the central limit theorem is better applied to repeated measurements than to an estimated set of parameters that are possibly correlated. The procedure was repeated one thousand times in order to constitute a statistically reliable data set and minimize the uncertainties in the estimated standard errors.

Figure 4 shows the statistical fluctuations in the resultant values of α and β which cannot explain the results of one of the experiments having coordinates (0.28,0.03). One can say that this large deviation is due to a change in outer normal conditions ('Gremlin' error), a variation in the radiosensitivity of the cell line or both. A huge amount of 'Gremlin' error cannot be assumed since a great effort was made to keep the same conditions for all the experiments. On the other hand, the four experiments were performed over a period of five months, and this flawed experiment happened to be first one. Therefore, one can conclude that a combination of the two above sources of error may have affected the results.

As shown in figure 5, the radiosensitivity parameters derived from the four experiments show a great variation which is a reflection of the variation seen in figure 3, namely from the α and β values. In order to estimate the true statistical variation, we use the values obtained from simulation since four experiments do not constitute a statistically sufficient

sample, and we have doubts about one of the experiments might have had additional errors other than statistical.

The coefficients of variation shown in table 2 indicate a huge variation in the α/β ratio (70.9%) compared to the other 2 parameters. This can be explained using the value of the correlation coefficient of α and β in table 1. Noting that ρ falls between [-1 to 1], the value found here (-0.9626) indicates a high degree of anticorrelation that exists between these 2 parameters. Therefore, the use of this parameter as a predictor of radiosensitivity is extremely dangerous since it fluctuates within large range of uncertainty.

The mean inactivation dose ($\bar{D}$) shows the least variation (6.9%). This is consistent with other experimental results which showed a variation of 5% for Chinese hamster V79 cells over a period of roughly 1 year³⁰.

On the other hand, SF2 shows a slightly higher variation (8.5%) which is contrary to what was found experimentally for the C3H10T1/2 cells based on 8 survival sets of data³¹. In the former study the α/β ratio was found to vary dramatically (from 1 to 17 Gy) from experiment to experiment. The smallest variations were found in $\bar{D}$ and SF2 (16 and 15%, respectively). These results are not surprising since they are based only on 8 experiments, possibly from different laboratories.

The real challenge comes from a simulation work which concluded that SF2 is a more reliable and numerically more stable parameter of cell radiosensitivity than $\bar{D}$. The

coefficients of variation of $\bar{D}$ and SF2 were 6.2 and 1.1% respectively³².

The main difference between the above simulation and the current one comes from the source of statistical variation considered. In the above simulation, the Binomial distribution variance was the only source of error considered. However, when considering an error contribution from sampling the plated cells, the survival variance will increase. The relative increase in survival variance (relative to the survival) is high in the range of low doses where the number of sampled cells is small (about 100 cells). At high doses the number of plated cells is about 10^5 and hence sampling is done with less uncertainty.

Thus, the relative variation of sampled cells, and hence the variation of the survival, decreases with increasing amount of sampled cells or dose used. Therefore, the inclusion of this term will allow greater fluctuations in the low dose survival, and hence make the above estimate of variation in the SF2 (1.1%) an underestimation. Moreover, the number of cell survival curves generated in the above study is relatively small (50) compared to the present one (1000).

On the whole, cell survival curves are well described by the fundamental parameter of the inactivation probability density. It is found that the value of parameter $\bar{D}$ is the least variable from experiment to experiment compared to those of SF2 (although they are highly correlated, figure 6) and

α/β , a behavior seen in experiments and supported by simulation.

In the attempt to compare the above models, special attention was paid to the extrapolation to zero dose. This was accomplished with the aid of the plating efficiency parameter introduced earlier. The results of the four experiments were normalized based on this parameter and averaged afterwards. Therefore, the fitting procedure not only measures how closely the model fits the data but also the way the model takes advantage of the data and predicts the behavior at zero and large doses.

The comparison of the four models shows that the Saturable Repair model gives the most accurate fit to the data. This is seen from its highest Q value. On the other hand, the Lethal-Potentially Lethal model shows the worse fit although the Q value is still acceptable. The Linear Quadratic and the Linear Quadratic-Cubic models show very close results; however, they differ drastically in their extrapolations.

Similar results were reported when six models were tested for their goodness of fit of 53 survival curves for human cells irradiated in plateau phase after completion of repair of potentially lethal damage. The Saturable Repair model gave the most accurate description of the data. The Linear Quadratic model had the most reliable parameters in terms of resolvability and robustness ²⁹.

On the other hand, as mentioned in the introduction, survival curves tend to have a nonzero slope at zero dose. In fact, using a more accurate assay such as the microscopic or the cell sorting assay, it was possible to see the behaviour of survival curves at low doses ($\ll 1$ Gy). This assay, which removes much of the random error associated with dilution and plating, was used to obtain survival curves for many cell lines. The results reported have shown that the LQ model underestimates the initial slope of survival^{33, 34}.

The results of fitting the four models (figure 8) show that indeed the LQ model predicts the second highest curvature at low doses. However, this behaviour is altered by introducing the LQ Cubic model. The SR model predicts a similar response to the LQ cubic. Together, the SR and the LQ Cubic model predict a continuously bending curve at high doses which is not in accord with an exponential final slope^{15, 35}.

Summing up, if an accurate description of the survival data is sought, then the Saturable Repair model ought to be used. The LPL model showed the worst behaviour. When reliability of parameters is required, the LQ model and, even more, the LQ Cubic model are the best. This latter result is important since the parameter estimates of the LQ model are needed by the "incomplete repair" model³⁶ (discussed in the next chapter) to describe the survival curve response after fractionated irradiation.

Table 1: Linear Quadratic model parameters.

LQ model	α (Gy ⁻¹)	β (Gy ⁻²)
estimates	0.0775	0.0828
std. errors	0.0519	0.0050
correlation coefficient*: $\rho = -0.9626$		

* The correlation coefficient relates α and β , and derived from the simulation experiment

Table 2: Coefficients of variation of radiosensitivity parameters of the LQ model derived from simulation. σ is the standard deviation of the parameter considered.

$\frac{\sigma_{\alpha/\beta}}{\alpha/\beta}$	$\frac{\sigma_D}{D}$	$\frac{\sigma_{SF2}}{SF2}$
70.9%	6.9%	8.5%

Table 3: Fitness and radiosensitivity parameters derived from the 4 models

	χ^2 *	$Q^\dagger$	SF2	$\bar{D}$ (Gy)
LQ model	21.34	0.045	0.615	2.663
LPL model	25.56	0.004	0.626	2.699
SR model	17.44	0.095	0.566	2.562
LQC model	20.00	0.045	0.554	2.514

* Residual square resulting from the fit (minimum)

† Goodness-of-fit coefficient

Figure 1: Growth curve, medium changed on days 4 and 7. Cells were grown in T-25 flasks. Error bars represent the standard deviation of three counts.

Figure 2: Continuous HDR (1.12 Gy/min) irradiation treatment. Raw survival data of one of the experiments which are fitted by the LQ model.

Figure 3: Linear-Quadratic parameters. Results of four independent experiments. The central point with error bars constitutes the average of the four experiments.

Figure 4: Linear-Quadratic parameters. Overlay of the results of 4 independent experiments and 1000 simulation experiments which are based on the resulting parameters of the four. Experimental data parameter estimates that cannot be seen are covered by simulated ones.

Figure 5: Experimental alpha/beta vs SF2 (upper axis, diamonds) and mean inactivation dose (lower axis, triangles).

Figure 6: Simulated alpha/beta vs SF2 (upper axis, diamonds) and mean inactivation dose (lower axis, triangles).

Figure 7: Resultant survival curve fitted by the four models. The goodness of fit is given for each fit.

Figure 8: Overlay of the four fits starting with the LPL model as it shows the highest survival. The inset shows the behavior of every model in the low dose region.

3.0 CONTINUOUS LOW DOSE RATE AND FRACTIONATED HIGH DOSE RATE IRRADIATION TREATMENTS

3.1 INTRODUCTION

It is observed that the dominant factor on which success or failure in clinical radiotherapy depends is recovery from radiation damage. Splitting a given total dose into many fractions or giving that dose in a low dose rate fashion allows repair after each fraction or during irradiation. After all, low dose rate irradiation is the limiting case of increased fraction number and reduced dose per fraction. It allows a differential recovery from radiation damage between normal and malignant tissues³⁷.

Many models exist for the description of fractionated or low dose rate treatment response^{38,39}. The most important is the "incomplete-repair" model of Thames³⁶ which laid the foundation for the analysis of experimental data relating to sublethal damage (SLD)⁴⁰. Moreover, it proved to have a relation to generally acceptable theories.

The "Incomplete-Repair" model

Oliver⁴¹ observed that the increase in dose required from a low dose rate exposure to achieve the same effects as high dose rate could be explained by the concept of a "dose-equivalent of incomplete repair" in a split-dose experiment. However, Oliver's model of survival remained empirical in

nature with many questions relating to multiple fractions regimens.

Hence, this model was initially derived for survival after two acute doses of size x and separated by a given time interval. After giving the second dose, an initial segment of the survival curve equivalent to a θx dose effect (where $0 < \theta < 1$) is not repeated, and therefore it is described by the repetition of the rest of the first segment. The mathematical description of survival after two doses of size x was given by

$$\ln(S) = \ln f(x) + \ln \left[\frac{f(x + \theta x)}{f(\theta x)} \right] \quad (57)$$

where $f(x)$ is the surviving fraction after dose x and θ is the repair kinetics which is considered to have a simple exponential form:

$$\theta = \exp(-\mu \Delta T) \quad (58)$$

where ΔT is the time interval between the two given doses and μ is the repair kinetics parameter which is related to the repair half-time ($T_{1/2}$) by

$$\mu = \frac{\ln 2}{T_{1/2}} \quad (59)$$

Equation (57) can not be simplified or expanded without using a model for $\ln f(x)$ that is linear in its parameters. Because the Linear Quadratic model adequately represents survival data, it was adapted to represent $\ln f(x)$, giving

$$\ln f(x) = -\alpha x - \beta x^2. \quad (60)$$

Generalisation of the above model to fractionated exposures can be summarized as follows: after a third dose x , given after time ΔT from the second dose, the dose equivalent of incomplete repair is $\theta x + \theta^2 x$. For n fractions the logarithmic survival will be

$$\begin{aligned} \ln S(x, \theta) = & \ln f(x) + \ln \left[\frac{f(x + \theta x)}{f(\theta x)} \right] + \ln \left[\frac{f(x + \theta x + \theta^2 x)}{f(\theta x + \theta^2 x)} \right] + \dots \\ & + \ln \left[\frac{f(x + \theta x + \dots + \theta^{n-1} x)}{f(\theta x + \theta^2 x + \dots + \theta^{n-1} x)} \right]. \end{aligned} \quad (62)$$

Expanding the logarithmic functions in power series yields

$$\ln S_n(x, \theta) = n(-\alpha x - \beta x^2) - 2\beta x^2 \sum_{k=0}^{n-1} \sum_{i=1}^k \theta^i. \quad (63)$$

After carrying out the above summations, the final result is given by

$$\ln S_n(x, \theta) = n(-\alpha x - \beta x^2) - n\beta x^2 h_n(\theta), \quad (64)$$

where

$$h_n(\theta) = \left(\frac{2}{n} \right) \left[\frac{\theta}{1-\theta} \right] \left[n - \frac{1-\theta^n}{1-\theta} \right]. \quad (65)$$

The above two equations represent the IR model for fractionated irradiation after n doses of size x with

intervals ΔT between doses.

The usefulness of the above approach is that it can be extended to predict the response after low dose rate continuous irradiation. Using the definition of an equivalent low dose rate irradiation: that is when x and ΔT are allowed to tend toward zero as n tends toward infinity, such that the dose given continues to be $n.x$ and the time of irradiation (given by $(n-1)\Delta T$) remains constant, the total dose given will be

$$D = vt \quad (66)$$

where v is the dose rate and t is the irradiation time. Moreover, x can be replaced by

$$x = v\Delta T = \frac{vt}{n-1}. \quad (67)$$

Now, the limit of $\ln S_n$ (equation (64)) is sought as $\Delta T \rightarrow 0$ ($n \rightarrow \infty$). At this large limit for n , $n-1$ can be replaced by n in equation (67). Therefore, using x as vt/n , the expression for low dose rate survival will be

$$\ln S(vt, \mu) = -\alpha(vt) - \beta(vt)^2 g(\mu t), \quad (68)$$

where

$$g(\mu t) = \frac{2}{(\mu t)^2} [\mu t - 1 + \exp(-\mu t)]. \quad (69)$$

Summing up, the idea is that the injury induced by some fraction θ of each dose of size x is not repaired when the second dose is given after time interval ΔT . For more

fractions, the θ term increases in a geometric series fashion. It is assumed that θ decays exponentially with time, a representation of the idea of a "monoexponential" repair kinetics.

The IR model is successful in describing the results of experiments in which *in vivo* colony survival is assayed after multifractionated regimens with variable intervals, some "too short" for complete repair. The model can be used to describe response to continuous low dose rate exposures, in which setting it is equivalent to the Linear Quadratic model with a factor to allow for dose rate⁴² and to the "accumulation" model⁴³. Moreover, it has been shown to be equivalent to the LPL model after the latter has been generalized to an arbitrary number of fractions and to low dose rate exposure³⁶.

However, the application of the IR model has revealed many problems. It has been shown that the apparent sublethal damage repair rate during fractionated irradiation depended upon the fraction size^{44, 45}. Also, disparate α/β ratios were calculated during fractionated irradiation. The α/β ratio appeared to depend both upon the fraction size and interfraction time⁴⁴. Although this may reflect saturation of the repair process, it is not possible to rule out the existence of multiple repair processes differing in their kinetics.

In fact, it has been shown that the apparent α/β ratio, calculated assuming a single repair process, will appear to be

protocol dependent if SLD is, in reality, dependent upon more than one repair rate process³⁸. Moreover, studies measuring the repair of DNA double strand breaks have revealed bi-phasic repair kinetics⁴⁶. Therefore, it was a logical step to generalize the IR model to incorporate two independent rates of repair⁴⁷. Here, repair is assumed to proceed initially according to a fast exponential process, and to gradually slow at longer times to proceed according to a slow exponential process. Analytically, it has the following form

$$\ln S = -n(\alpha x + \beta x^2) - n\beta x^2 [\rho h_n(\theta_1) + (1-\rho) h_n(\theta_2)] \quad (70)$$

where h_n is given by equation (66), θ_1 and θ_2 are the two repair kinetics forms (equation (58)), and ρ determines the relative importance of each of the 2 repair processes. For continuous exposures, equation (68) is modified to

$$\ln S = -\alpha vt - \beta (vt)^2 [\rho g(\mu_1 t) + (1-\rho) g(\mu_2 t)] \quad (71)$$

where again μ_1 and μ_2 follow equation (59).

It is apparent that 2 additional parameters are required to account for an extra component of repair. This proves seriously disadvantageous in practice, since a small number of survival end points is not sufficient for accurate estimation of the model parameters⁴⁷.

The above form of the IR model was used for a reanalysis of an extensive data set and the results indicated the presence of two significantly different repair rates (a fast

and a slow one) and an α/β value that is consistent among complete and incomplete repair fractionation experiments⁴⁸.

The use of the LQ Cubic model in the IR model

As derived in chapter 2, the Linear Quadratic cubic model has shown real promise, especially in the estimation of the initial slope. In order to cover other possibilities, the IR model is now rederived based on the LQ cubic model. Therefore, replacing $\ln f(x)$ in equation (60) by

$$\ln f(x) = -ax - bx^2 - cx^3 \quad (72)$$

and expanding equation (63), we will get

$$\ln S_n = n(-ax - bx^2 - cx^3) - (2bx^2 + 3cx^3) \sum_{k=0}^{n-1} \sum_{i=1}^k \theta^i - 3cx^3 \sum_{k=0}^{n-1} \left(\sum_{i=1}^k \theta^i \right)^2 \quad (73)$$

The first double sum was evaluated in the IR model derivation. The second double sum is now evaluated using the result of an arithmetic-geometric series closed form; therefore,

$$\sum_{k=0}^{n-1} \left(\sum_{i=1}^k \theta^i \right)^2 = \left[\frac{\theta}{1-\theta} \right]^2 \left[n - \frac{2(1-\theta^n)}{1-\theta} + \frac{1-\theta^{2n}}{1-\theta^2} \right] \quad (74)$$

and the final form of $\ln S$ will be

$$\ln S_n = n(-ax - bx^2 - cx^3) - n(bx^2 + \frac{3}{2}cx^3) h_n(\theta) - 3cx^3 z_n(\theta) \quad (75)$$

where $z_n(\theta)$ is equivalent to the identity in equation (74).

For low dose rate exposure, the previous limits are applied and $\ln S$ will be given by

$$\ln S(vt, \mu) = -a(vt) - b(vt)^2 g(\mu t) - c(vt)^3 R(\mu t) \quad (76)$$

where $g(\mu t)$ is given by equation (69) and $R(\mu t)$ is found to be

$$R(\mu t) = \frac{3}{(\mu t)^3} \left(\mu t - \frac{3}{2} + 2e^{-\mu t} - \frac{e^{-2\mu t}}{2} \right). \quad (77)$$

Derivation of a survival model after PLDR

The previous discussion is related to the repair of sublethal damage (SLD). If cells are left to repair potentially lethal damage (PLD), what sort of curve will the survival follow, and more interestingly, what repair kinetics will this type of repair process take?

Several attempts have been made to understand whether SLD and PLD are the same or different, qualitatively and / or quantitatively, from each other⁴⁹. In order to predict the survival after PLDR, we approximate the repair process by monoexponential repair model, according to which the rate of repair is proportional to the expectation value of the number of lesions per cell to be repaired:

$$\frac{dN}{dt} = -\mu N \quad (78)$$

where now μ is the PLD repair kinetics parameter. At any time during repair, N is the total number of lesions present minus the number of irreparable lesions. That is

$$N = N_{total} - N_{residual} \quad (79)$$

where the residual lesions are those still present after an infinite amount of time. Substituting for N in equation (78)

yields

$$\frac{dN_t}{dt} = -\mu (N_t - N_r) \quad (80)$$

where N_r is considered to be constant throughout the repair process. Assuming that the initial total number of lesions is N_{t0} , at zero time, the total number of lesions at a time t is found by integrating equation (80);

$$N_t = N_r + (N_{t0} - N_r) e^{-\mu t}. \quad (81)$$

Assuming lesions are distributed according to Poisson statistics in the cell, the survival at a time t will be given by

$$S(t) = e^{-N_t} = e^{-N_r - (N_{t0} - N_r) e^{-\mu t}}. \quad (82)$$

Following the same assumption, we can substitute

$$S(0) = e^{-N_{t0}} \quad (83)$$

and

$$S(\infty) = e^{-N_r} \quad (84)$$

in equation (82) to get the final form of the survival after a time t of repair of PLD:

$$S(t) = S(0) \left\{ \frac{S(\infty)}{S(0)} \right\}^{(1 - e^{-\mu t})}. \quad (85)$$

In this chapter we examined the kinetics of SLDR after

continuous low dose rate and fractionated high dose rate irradiation. We also examined the kinetics of PLDR after 3 Gy of high dose rate irradiation. The specific objectives are: to fit the IR model in its original form, in its 2 repair rates form, and in its new derived form based on the LQ Cubic model; to determine from the above three forms repair half time values and examine their behavior as a function of dose per fraction; to determine whether the kinetics and the mechanisms of SLDR and PLDR are similar.

3.2 MATERIALS AND METHODS

The human glioma cell line U-87MG was cultured as described in section 2.2. For all experiments 4×10^3 cells per cm^2 were plated in 25 cm^2 flasks for HDRI and in 1.3 cm^2 glass vials for LDRI. The medium was changed (4 ml in flasks and 0.5 ml in vials) on days 4 and 7 post-plating with experiments performed on day 10.

For continuous LDRI, cells were irradiated by an array of ^{226}Ra sources (total activity 800 mCi) contained in a temperature controlled incubator. Two low dose rates were used, LDR1 at 0.88 cGy/min and LDR2 at 0.41 cGy/min. These dose rates were determined by TLD dosimetry relative to a ^{60}Co reference protocol²³. Irradiation were performed at 37°C (± 0.1 C) monitored by thermocouple placed in the irradiation chamber.

For fractionated HDRI, cells were irradiated as described in section 2.2 and placed, medium unaltered, in 37 °C water bath for the required interfraction interval ΔT . This procedure was also followed when giving single dose fraction (PLDR) where now t is employed instead of ΔT .

Cell survival was assayed by the colony formation assay (section 2.2).

Most data points represent the average of 2 independent experiments. However, some of them represent 3-4 independent experiments. Fitting procedures were applied as described in section 2.2.

3.3 RESULTS

For LDRI treatment, figure 9 shows a growth curve of cells in glass vials. Continuous LDR1 (0.88 cGy/min) and LDR2 (0.41 cGy/min) irradiation survival curves are shown in figure 10. They were fitted to the IR model in its low dose rate form (equation (68)). Also shown is the continuous HDR (1.12 Gy/min) irradiation survival curve obtained from the LQ model (figure 8).

Fractionated HDRI survival curves using various dose per fraction regimens are presented with an overlay of the 2 LDR curves serving as a reference survival level. The fractionated curves are grouped as a function of the dose per fraction used to show the effect of interfraction recovery time without adding a third variable.

The choice of interfraction intervals is limited by three factors: the maximum allowed time for a plateau phase experiment which extends to a maximum of 3 days as shown in figure 1, the maximum amount of total dose that can be given (around 10-12 Gy of HDRI) which is determined by the precision of the colony formation assay (above 10 Gy) and the experimenter tolerance: for instance, giving fractionated HDRI of 1 Gy every 3 hr requires 30 h of continuous work in order to go up to 10 Gy of total dose.

Figure 11 shows the results of giving 0.5 Gy every 1 hr. The 1, 1.5, 2, 3 and 4 Gy per fraction protocols are shown in figures 12, 13, 14, 15 and 16, respectively. All the fits

presented in these figures were derived from the IR model in its original form (equation (63)). The biphasic repair (equation (70), (71)) and the Cubic (equation (75), (76)) form of the IR model were also fitted to the same set of data. A chi-square test was applied in all cases and the total sum of residuals from all the curves is presented in table 5 for the 3 models.

The repair rate values (or kinetic coefficients) (μ) estimated with the original form of the IR model are plotted in figure 17(a) as a function of dose per fraction. The zero dose per fraction repair rate is estimated from the LDR2 survival data which constitutes the lowest dose per fraction given. We did not observe a significant difference in the behavior of the repair rates when fitting the IR Cubic model. The results are shown in figure 17(b). Figure 18 shows an overlay of the repair rates from figure 17. A similar behaviour is observed although the 2 curves are shifted. The repair half time values ($T_{1/2}$) are presented in figure 19 following equation (59).

The two repair rates estimated with the IR biphasic form are presented in table 4. The estimates show large fluctuations especially in the fast repair rate μ_2 . Survival curves of 3 Gy and 4 Gy dose per fraction were not included in this analysis as they lack sufficient data points. No attempt was made to estimate the standard errors since they can not explain the noted variations. However, Table 5 shows that the

IR biphasic gave the smallest sum of square residual relative to the number of degrees of freedom used. These large residuals, however, will be discussed in the next section.

Finally, PLDR after single 3 Gy and fractionated 9 Gy are shown in figure 20. The choice of 8 hr interval is based on the assumption that all SLDR is complete. After that, it will be possible to assess PLDR after multiple fractions and compare it to SLDR fractionation protocols. Figure 21 shows an extrapolation of the PLDR Survival model (equation (85)) which predicts no significant increase in survival after 8 hr of incubation time. Table 6 shows the repair rate values of the 2 PLDR protocols which seem identical.

On the other hand, applying the IR model to 3 Gy every 8 h fractionation experiment showed a zero value for the repair kinetics θ (equation (58)) which means that there is a complete repair of SLD occurring during the 8 h interval. These results are consistent with the PLDR kinetics and previously published data²³.

3.4 DISCUSSION

It is well known that a recovery from sublethal radiation damage occurs during LDR and fractionated HDR irradiation treatments. Our results show that this recovery process depends on the protocol followed. Data on recovery kinetics of SLD showed a linear increase in repair half time with dose (after 1 Gy), an indication of a repair saturation process⁵⁰.

Other data derived from the same current cell line and in our laboratory showed again a linear increase in the repair half time values of PLD with dose (starting from 3 Gy)²³.

Furthermore, in split-dose and low dose rate experiments performed on 4 experimental tumour systems, repair half time values were found to be longer in split dose (5 + 5 Gy and 6 + 6 Gy) than in low dose rate (between 1.6 cGy/min and 150 cGy/min) experiments⁵¹. Therefore, we can say that our results (figure 19) are consistent with the latter ones. However, we note that none of the above references did go below 2 Gy as a dose per fraction. Below 1 Gy of dose per fraction, our data show a rise in the repair half time values as if the repair system is not as active as in the case of higher doses per fraction.

As discussed in section 2.4, a similar behaviour was seen in the case of single dose irradiation (from 0 to 1 Gy) where a more accurate assay was used. This phenomenon was called an induced resistance since survival at zero dose showed a very steep initial slope and at about 1 Gy the survival went up to follow what is predicted by the LQ model^{33,34}.

Therefore, in the context of an induced resistance we can explain the behaviour of repair half time values seen in figure 19 from zero to 1 Gy. After that, a repair saturation process might have taken place.

Even when we applied the IR Cubic model where the survival at low doses (predicted by the LQ Cubic) is more

certain, a similar behaviour was observed. The 2 curves in figure 19 are parallel. Since the IR Cubic model shows a moderately smaller total sum of residual squares than the IR model (table 5), it can be in the worse cases equivalent to the latter model since both of them use the same number of degrees of freedom.

The high value of the total residual squares resulted from the fact that repeated experiments were fitted by considering all the points without going back to the averaging technique discussed previously. This approach must produce the same estimates (compared with the averaged repeated experiments) since we are using a weighted least square method. However, the sum of residual square of repeated experiments fitted simultaneously is expected to be larger than that of the individual experiments fitted separately.

It is worth noting that the IR and the IR Cubic are supplied with parameters (α , β , and a , b , c) estimated in chapter 2. All the data presented in this chapter are used to estimate one parameter for every model per dose per fraction set. The IR biphasic model uses α and β quoted from chapter 2 as well.

On the other hand, the IR biphasic model has 3 parameters which are highly correlated. It is not surprising to see a big reduction in the total sum of residual squares as a result of those 2 additional parameters. However, the scatter in the 2 repair kinetics in table 4 can not be explained even in the

light of a strong correlation among the parameters. Furthermore, LDR1 protocol did not show 2 distinct repair kinetics.

One may say that more data are needed to show 2 coherent repair kinetics in the case of the IR biphasic model. Requiring more data than what is presented, however, will make the former model lose its practicality.

On the other hand, the PLDR experiments showed that even after multiple fractions of 3 Gy the repair process maintains its kinetics (table 6). Unfortunately, we can see that our results lack precision due to the small number of data points used which results in large uncertainties in the estimated kinetics. Constant repair rates after multiple fractions were confirmed in the case of SLDR fractionation experiments *in vivo*^{52,53}. However, a decrease of ability of 10T1/2 cells to repair PLD *in vitro* as the number of fractions increased (using 12 h interval) was reported^{54,55}. Thus, more data and various dose per fraction regimens are needed to support the current results.

Furthermore, our results do not rule out an equivalence between 3 Gy PLDR and 3 Gy dose per fraction SLDR kinetics (table 6 and figure 18). In fact, there is a 3 % difference between the 2 kinetics and the new derived PLDR model shows a complete repair after 8 hr of incubation time. Interestingly, we can show a relation that exists between the PLDR model and the IR model. Applying the IR model in the case of 2 fractions

separated by an interval ΔT (split-dose experiment, $n=2$), the survival will be

$$\ln[S(\Delta T)] = -2\alpha x - 2\beta x^2 - 2\beta x^2\theta. \quad (86)$$

Equation (86) can be rearranged to give

$$S(\Delta T) = e^{-2\alpha x - 4\beta x^2} \cdot e^{2\beta x^2(1-\theta)} \quad (87)$$

The survival when ΔT is zero is equivalent to giving 2 x dose at once which can be described by the LQ model:

$$S(0) = e^{-\alpha 2x - \beta 4x^2}, \quad (88)$$

and the survival when the 2 doses of x are separated by an infinite amount of time is the square of the individual effect. Hence

$$S(\infty) = e^{2(-\alpha x - \beta x^2)}. \quad (89)$$

Going back to equation (87) and using equations (88) and (89), we will have the final form of a split-dose experiment model:

$$S(\Delta T) = S(0) \left\{ \frac{S(\infty)}{S(0)} \right\}^{(1-\theta)} \quad (90)$$

which is identical to equation (85) which describes PLDR recovery. Expanding the exponentials in equation (90) in power series and taking terms to first order, we will end up having an equation identical to what it has been used previously for PLDR recovery^{56, 57, 23}.

Finally, 3 Gy PLDR and SLDR are shown to have the same

kinetics. Moreover, their underlying models are proved to be identical which supports the idea that PLDR and SLDR do not differ in their molecular mechanisms⁴¹.

On the whole, the IR model in its 3 forms is applied to continuous low dose rate and fractionated high dose rate irradiation. From the survival level noted in those experiments, it is found that LDR1 survival can be well simulated by 1 Gy/ 1 hr , 1.5 Gy/ 3 hr or 2 Gy/ 4 hr. These results, from a biological prospective, show that repair processes saturate while increasing the size of dose per fraction with the need of a longer time for repair (confirmed more rigorously by the analysis of variation of repair half time values). From a clinical perspective, they open the door for the use of an alternative technique for the low dose rate interstitial implant brachytherapy as it is less convenient to apply in some cases as discussed in chapter 1.

Table 4: Biphasic exponential repair kinetics estimated parameters.

	$\mu_1(\text{hr}^{-1})$	$\mu_2(\text{hr}^{-1})$	ρ^*
LDR2	0.088	19.77	0.3
LDR1[*]	0.255	0.256	0.2
0.5 Gy/frac	0.018	22.20	0.2
1.0 Gy/frac	0.153	11.37	0.4
1.5 Gy/frac	0.047	1.500	0.3
2.0 Gy/frac	0.245	22.26	0.6

LDR2 = 0.41 cGy/min

LDR1 = 0.88 cGy/min

^{*} the relative importance of μ_1

* LDR1 showed no indication of a biphasic exponential repair

Table 5: Summary of all the fitting procedures done using the 3 models. Numbers indicate the sum of residual square. The total square residuals and number of degrees of freedom for every model fitting are presented at the end.

	IR	IR Cubic	IR biphasic
LDR2	19.73	19.50	18.16
LDR1	106.2	106.6	140.3
0.5 Gy/frac	40.32	37.86	20.82
1.0 Gy/frac	122.5	123.1	71.63
1.5 Gy/frac	110.4	112.0	22.39
2.0 Gy/frac	305.5	297.3	238.7
Total Res.	704.6	696.4	512.0
D.o.f.	172	172	160

Total Res. = total sum of residual-square for all the experiments

D.o.f. = number of degrees of freedom

Table 6: PLDR kinetic parameters

	$\mu(\text{hr}^{-1})$	Std. Error*	Q-value
Xt	0.387	0.240	0.744
X8X8Xt	0.381	0.054	0.028

* Standard Error estimated from constant probability contour where it is assumed that the parameters are uncorrelated (See the Appendix).

Q-value = goodness-of-fit coefficient

X = 3 Gy

t = incubation time at 37°C, 8 = 8 hr at 37°C

Figure 9: Growth curve, medium changed on days 4 and 7. Cells were grown in glass vials (1.3 cm²).

Figure 10: Continuous LDR1: IR model fit to LDR1 (0.88 cGy/min) and LDR2 (0.41 cGy / min) irradiation treatment. HDR survival is presented by the LQ model (from figure7).

Figure 11: Fractionated HDR: 0.5 Gy/ 1 hr. LDR1 and LDR2 survival are also shown.

Figure 12: Fractionated HDR: 1 Gy/ 1 hr and 1 Gy/ 2 hr fit to IR model. LDR1 and LDR2 survival are also shown.

Figure 13: Fractionated HDR: 1.5 Gy per fraction at various interfraction intervals fit to the model. LDR1 and LDR2 survival are also shown.

Figure 14: Fractionated HDR: 2 Gy per fraction at various interfraction intervals fit to IR model. LDR1 and LDR2 survival are also shown.

Figure 15: Fractionated HDR: 3 Gy per fraction at various interfraction intervals fit to IR model. LDR1 and LDR2 survival are also shown.

Figure 16: Fractionated HDR: 4 Gy per fraction at 2 hr interfraction interval fit to IR model. LDR1 and LDR2 survival are also shown.

Figure 17: Repair rate constant : (a) Results of fitting the IR model. (b) Results of fitting the IR Cubic model.

Figure 18: Repair rate constant: Results of fitting the IR and the IR Cubic models.

Figure 19: Repair half-time : Results of fitting the IR and the IR Cubic models.

Figure 20 : PLDR Survival : Survival following a delayed plating period at normal temp.

(a) Survival after 3 Gy dose. (b) Survival after 3 doses of 3 Gy separated by 8 hr interval.

Figure 21: PLDR Survival: extrapolation of the PLDR model to 24 hr incubation.

4.0 FRACTIONATED HIGH DOSE RATE IRRADIATION COMBINED WITH MILD HYPERTHERMIA TREATMENT

4.1 INTRODUCTION

Hyperthermia alone is not yet established as a definitive form of cancer therapy, comparable to radiotherapy or chemotherapy. Local tumours can be sterilized efficiently at high temperatures ($> 45^{\circ}\text{C}$), which cause significant damage to surrounding normal tissues¹¹. Mild hyperthermia (41°C), however, in combination with radiotherapy has been shown to be more effective than either modality alone, especially, in the case of low dose rate irradiation. Therefore, concurrent mild hyperthermia combined with LDRI used in brachytherapy can enhance the effectiveness of clinical brachytherapy treatments^{58,59}.

At temperatures greater than 42°C hyperthermia inhibits the repair of radiation-induced strand breaks and radiation-induced chromosome aberrations. This inability to repair molecular damage translates into the inability to repair both sublethal damage (SLD) and potentially lethal damage (PLD) produced by radiation^{9, 11}.

The SLD recovery curve for a split dose experiment shows an initial steep climb after which a plateau is reached. The shapes of such curves suggest exponential functions, and this resulted in the notion of monoexponential repair kinetics⁴⁷.

Fractionated irradiation is a general form of a split

dose experiment. Therefore, assuming that the repair process is a first order enzyme kinetics, the rate of repair of lesions at normal temperature (37°C) is given by

$$\frac{dN}{dt} = -\mu N \quad (91)$$

where N is the number of lesions to be repaired. Therefore, the fractional number of lesions (relative to the initial number present directly after the radiation dose) decreases according to the function

$$\exp(-\mu t) \quad (92)$$

which is the monoexponential repair kinetics θ used in the "incomplete repair" (IR) model discussed previously⁴⁷.

On the other hand, many heat survival curves have a shoulder. It has been postulated that the shoulder implies an ability to sustain sublethal damage which led to the use of a radiation target theory model in the analysis of heat survival curves⁶⁰. However, it has not been possible to show by experiment whether or not repair of heat sublethal or nonlethal lesions does occur in heated cells⁶¹.

The only mechanistic theory of cellular inactivation by heat has been proposed by Horst Jung^{61, 62, 63} and a generalized concept has been developed. The basic idea of this concept is that cellular inactivation by heat is a two step process. In the first step, heating produces nonlethal lesions. In the second step, the nonlethal lesions are converted into lethal events upon further heating. The

conversion of one of the nonlethal lesions in a cell leads to cell death.

Based on the assumption that both production and conversion of nonlethal lesions occur at random, that is the number and the conversion of nonlethal lesions per cell is distributed according to Poisson statistics and depend only on temperature, a mathematical model was worked out which gives the survival as a function of heating time^{51, 52, 53}:

$$S(t) = \exp\left(\frac{p}{c} [1 - ct - e^{-ct}]\right) \quad (93)$$

where p is the rate constant for the production of nonlethal lesions per cell and per unit time, and c is the rate constant for the conversion of one nonlethal lesion into a lethal event per cell per unit time. An important feature of these two parameters is that they depend only on the temperature of heat treatment.

One major problem that the above model faces is the possible occurrence of directly lethal lesions (in a single step). It cannot completely be excluded that, especially at high temperatures ($>45^{\circ}\text{C}$), a minor portion of the observed cell killing is caused by one-step formation of lethal lesions. However, all our experiments were done under mild hyperthermia ($<42^{\circ}\text{C}$). Thus, the use of the above model in interpreting our data is justified.

Derivation of a repair kinetics model under hyperthermia

Hyperthermia induction of increased radiation sensitivity has been observed in many cell systems studied. Heating has been applied before and after irradiation resulting in a synergistic effect, a phenomenon frequently termed as a thermoradiosensitization⁶⁴.

In order to describe quantitatively the effect of heat on radiation induced lesions, we employ the above 2 concepts of monoexponential lesion repair and heat lesion production. Using the Poisson assumption for the conversion of nonlethal lesions into lethal lesions under hyperthermia, we write the survival as the probability that a cell has no lethal lesions:

$$S(t) = \exp(-\Gamma) \quad (94)$$

where Γ is the mean number of lethal lesions converted per cell. Since equations (93) and (94) are equivalent, Γ will be

$$\Gamma = -\frac{D}{C} [1 - ct - e^{-ct}] \quad (95)$$

and the rate of formation of these lesions per unit of time can be presented as

$$\frac{d\Gamma}{dt} = p(1 - e^{-ct}) . \quad (96)$$

Considering the above equation, we can easily verify that c is the rate constant for the conversion into lethal lesions per cell per unit of time. The above rate goes to zero when

its constant (σ) is set to zero.

Therefore, giving a radiation dose and applying heat afterwards, we expect to observe an interaction between heat and radiation induced lesions. Let M be the expectation value of the total number of lesions that are unrepaired (after the radiation dose) and produced (by heat) at a given time t . Moreover, let M_0 be the expectation value of the number of lesions produced at the end of the radiation dose exposure. So M_0 is the initial value of M at the starting time of the repair and heat processes.

At a given time t (after the radiation dose) the rate at which M varies with respect to time is given as the resultant rate of repair (equation (91)) and the rate of heat lethal lesion formation (equation (96)):

$$\frac{dM}{dt} = -\mu M + p(1 - e^{-\sigma t}). \quad (97)$$

Dividing both sides of the equality by M_0 allows us to deal with ratio of lesions present. Therefore, denoting the latter ratio by H ($H \equiv M/M_0$), we can change equation (97) to another form that has H as the dependent variable:

$$\frac{dH}{dt} = -\mu H + p' (1 - e^{-\sigma t}) \quad (98)$$

where now p' replaces p/M_0 in the above equality.

The above equation is a first order linear differential equation. In order to solve it, we set another short notation by letting $I = \exp(\mu t)$. If we multiply H by I and take their

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derivative, we can get an expression equivalent to equation (98):

$$\frac{d(H.I)}{dt} = e^{\mu t} \cdot p' (1 - e^{-ct}) \quad (99)$$

and hence,

$$H.I = \int e^{\mu t} \cdot p' (1 - e^{-ct}) dt. \quad (100)$$

Solving the above integral, we find $H(t)$ to be

$$H(t) = p' \left(\frac{1}{\mu} - \frac{e^{-ct}}{\mu - c} \right) + E \cdot e^{-\mu t} \quad (101)$$

where E is a constant of integration.

A boundary condition can be imposed on equation (101). At zero time, the fraction of number of lesions ($H(0)$) is 1 (since repair and heat conversion have not taken place yet). Therefore, when t is zero H is set to 1. Using equation (101), E can be found:

$$E = 1 - \frac{p'}{\mu} + \frac{p'}{\mu - c} \quad (102)$$

and finally, H is given by

$$H(t) = \frac{p'}{\mu} - \frac{p'}{\mu - c} e^{-ct} + \left(1 - \frac{p'}{\mu} + \frac{p'}{\mu - c} \right) e^{-\mu t}. \quad (103)$$

The above repair kinetics can be interpreted as a biexponential function resulting from a monoexponential repair and a heat killing term. In the case when hyperthermia is not applied, we go back to equation (96) and set the rate of

conversion into lethal lesions to zero. This can be simply done by setting the rate constant c to zero. Thus, setting c to zero in equation (100), we get the limit value of $H(t)$:

$$H(t) = e^{-\mu t} = 0 \quad (104)$$

which corresponds to the monoexponential repair kinetics under normal temperature used in the "incomplete repair" (IR) model.

Furthermore, combining fractionated irradiation and mild hyperthermia (<42°C) quantitatively can be realized in the light of the above derivations. Using high dose rate fractionated irradiation, the repair kinetics between fractions under the effect of heat can be described by equation (103). Moreover, the survival end point can be still assumed to follow the (IR) model, since it is a function of the whole repair kinetics:

$$\ln S_n(x, H) = -n(\alpha x + \beta x^2) - n\beta x^2 h_n(H) \quad (105)$$

where h_n is given by equation (65) and H is now given by

$$H(\Delta t) = \frac{D'}{\mu} - \frac{D'}{\mu - c} e^{-c\Delta t} + \left(1 - \frac{D'}{\mu} + \frac{D'}{\mu - c}\right) e^{-\mu\Delta t}. \quad (106)$$

Similarly, applying the previously derived IR Cubic model, we get

$$\ln S_n = -n(\alpha x + \beta x^2 + \gamma x^3) - n\left(\beta x^2 + \frac{3}{2}\gamma x^3\right) h_n(H) - 3\gamma x^3 z_n(H) \quad (107)$$

where z_n is given by equation (74).

In this chapter we investigated the effect of combining or, more appropriately, sequencing high dose rate irradiation and hyperthermia at 41C and fractionated 43C for various dose per fraction protocols. The results of chapter 3 serve as control experiments when hyperthermia was not applied. Repair rates μ are estimated for every dose per fraction regimen and compared to the values found previously. Furthermore, repair kinetics values (μ) under hyperthermia are estimated for every survival curve to show the variation of survival level relative to the HDRI level.

4.2 MATERIALS AND METHODS

The cell line U-87MG was cultured as described in section 2.2. For fractionated HDRI and hyperthermia treatments, cells were grown in 25 cm² flasks (4x10³ cells per cm²). Hyperthermia treatment was accomplished by heating in water (± 0.05 C). Cell survival was assayed by the colony formation assay (section 2.2). Most experiments were repeated twice. Some of them, however, were repeated 3 times.

In the previous chapter, error estimation was performed on the repair half time values derived with the IR model. As the IR model was supplied with α , β , a , b , and c parameters, the repair rate (and hence the repair half time) was the only estimated parameter. Therefore, a one dimensional root finding routine (Zbrent)²⁸ was used to estimate the interval of the parameter variation while the minimum residual square was

increased by unity (see the Appendix).

In this chapter, fitting was accomplished with the aid of another minimization method which is based on the Levenberg-Marquardt method for models which are nonlinear in their parameters²⁸. This method and the previous one (following the method of Powell) were compared and proved to give the same estimates within a tolerance of 10^{-3} . The advantage of using this current method is its ability to estimate errors on parameters that are known to be correlated.

4.3 RESULTS

Hyperthermia alone was applied for the 2 cases of 41 and 43C (figure 22). The heating time was applied for up to 12 h for 41C and 4 h for 43C. The 12 h for the 41C experiment is chosen since it corresponds to the maximum treatment time used in combination with fractionated irradiation (in this study). The graph shows a moderate decrease in survival during this time period. On the other hand, the 43C curve shows a huge decrease in survival. The problem found with this treatment is the loss of cell ability to stay attached to the bottom of the flask. After 4 or 5 h at 43C, 50% of the cells started to float in the medium due to the denaturation of their plasma membrane proteins which serve as an attachment site⁶⁵. Only cells that were still attached were plated for survival.

The *p&c* model of hyperthermia (equation (93)) was used to fit both curves. An excellent fit is observed in the 2 cases

with a Q-value >0.7 . Furthermore, the plating efficiency was estimated with the aid of the latter model as discussed previously (section 2.2).

Fractionated hyperthermia was also performed to enhance the shallow survival of 41C and the acute steep response of 43C. Figure 23 shows the results of 2 independent experiments where a 15 min at 43C was given every hour. The time shown is the total time of 43C heating used to bring the survival to the indicated level. In the absence of a suitable model, these data were not normalized. However, relative to the first few fractions, there is a minimal decrease in the level of survival which is attributed to the thermotolerance effect seen after fractionated heating. This latter effect is a phenomenon in which cells become resistant to elevated temperatures as a result of prior or continuous exposure of hyperthermia⁵⁶. Therefore, the application of fractionated hyperthermia alone is proved to be disadvantageous even when using elevated temperatures.

Fractionated 43C hyperthermia was used for the case of 1 Gy every 1 hr dose per fraction regimen. A period of 15 min at this acute temperature was applied before every radiation fraction dose as shown in figure 24. Again, the data were not normalized. However, an HDRI curve based on the LQ model is plotted starting from the experimental zero dose point of these data.

Continuous 41C hyperthermia was applied during the

interfraction intervals of 1, 1.5 and 2 Gy dose per fraction regimens. Figures 25, 26 and 27 show the response for various fractionation intervals. The common behaviour is a decrease in the survival down to the HDRI level compared to the results of chapter 3. A net repair response is still observed while increasing the interfraction time intervals.

These data were fitted to the IR and the IR cubic models incorporating the new repair kinetics ($H(\Delta t)$) that takes into account the hyperthermic conditions. For every dose per fraction regimen the repair rate coefficient μ is estimated and the results are presented in tables 7 and 8. Statistical results are presented in table 9.

Equivalently, H values (for the IR and the IR cubic models) are estimated from each individual curve and shown in figures 28 and 29. Using the estimates of the 3 parameters μ , p' and c , $H(\Delta t)$ (equation (106)) is overlaid as a function of interfraction interval for 1 and 2 Gy dose per fraction. The magnitude of H determines the level of survival relative to the HDRI survival.

4.4 DISCUSSION

The $p\&c$ model has been developed and tested using data of the heat response of exponentially growing CHO cells⁶¹. It has been recently applied to analyze data on growth delay of a C3H mammary carcinoma *in vivo*⁶⁷. The present data, representing plateau phase cells of human origin, are the first of their

kind to be analyzed with this model. Our results show an excellent agreement within the heating time frame used.

Prolonged exposure to mild temperatures (40 and 41C) were also performed (data not shown) to analyze the effect of thermotolerance. The *p&c* model was previously extended to account for such an effect^{62, 63}. The idea was that *p* changes in an exponentially decreasing fashion as a function of increasing heating time. Unfortunately, an addition of 2 extra parameters made the extended model inappropriate for the analysis of these data. Consequently, the analytical description of fractionated heating remains an unsolved issue.

With regards to the problem of one step lethal lesion production at high temperatures, we can modify the *p&c* model to account for such effect. Assuming that the production of these lesions is linear with heating time (following the same approach of the LPL model (equation (19)) as in the case of radiation), equation (96) will be

$$\frac{d\Gamma}{dt} = p(1 - e^{-ct}) + q(T) \quad (108)$$

where *q(T)* is the rate constant of single step formation of lethal lesions and it is a function of temperature only. For low temperatures (<45C) this rate constant is assumed to decrease and become nonsignificant below 42.5C (when thermotolerance is initiated in many cell lines).

Integrating equation (108) and following the same approach as in equation (93), the survival will be

$$S(t) = \exp \left(qt + \frac{p}{c} [1 - ct - e^{-ct}] \right). \quad (109)$$

An interesting observation emerges from equation (109). Going back to the IR model in its low dose rate form, we can see that equation (109) and equation (68) are identical if we set the following equalities:

$$q = \alpha v,$$

$$p = \frac{2\beta v^2}{\mu},$$

$$c = \mu.$$

This equivalence between the two models can be explained in the light of similarities in their postulates. The IR model is based on the LQ model (section 2.1) which assumes that cell death results from a double strand break in the DNA resulting from one radiation event or two independent radiation events on each strand. On the other hand, the extended **p&c** model assumes that cell death results from one step heat killing event or 2 steps formed by the production of nonlethal lesion and its conversion into lethal one in a kinetically independent way.

Therefore, both models are built with the same logic. However, the proposed concepts of the **p&c** model cannot provide any evidence as to what these lesions may be.

The results of 41C hyperthermia combined with fractionated irradiation have shown real promise. The 1 Gy

every 2 hr regimen without hyperthermia (figure 12) showed a survival level between LDR1 and LDR2 level. While applying hyperthermia, the survival level has been brought down to the HDRI level. A similar effect is seen for the case of 1.5 and 2 Gy dose per fraction regimens.

It is customary to explain this effect as an inhibition of radiation SLDR^{8,11}. However, following the derivation of the hyperthermia repair kinetics (equation (103)) the above effect is the result of two competing rates, one for repair and the other for killing. It is true that the rate of killing by hyperthermia alone measured in figure (22) cannot explain the huge decrease in survival level after combining the two modalities. In fact, going back to the 2 step process of the *p&c* model, we can say that radiation did initiate the first step in the majority of nonlethal lesions present as a form of SLD while hyperthermia, with its entire killing capability, took care of the second step. However, a net repair of radiation damage is still observed.

The combined fractionated hyperthermia at 43C with radiation (figure 24) supports this idea as well. During the latter regimen cells were allowed to repair for 45 min and subjected to heat for 15 min. Therefore, SLDR got the chance to occur between the radiation fractions, yet the survival level resembles the HDRI level.

The evidence that supports the occurrence of a SLDR under hyperthermia (especially at this mild temperature) are the

estimates of the repair rate values (μ). As shown in tables 7 and 8 there is an acceptable agreement between values of repair rate estimated under normal and hyperthermia conditions within the range of uncertainty.

Another way of looking at this repair phenomenon is the individual values of the repair kinetics H estimated from every individual survival curve. Pointing out that a value of 1 for H indicates HDRI survival level, we can see that all the values found are below 1, suggesting a net repair process has occurred between dose fractions. Furthermore, the repair kinetics function $H(\Delta t)$ extrapolates to a constant value indicating that a further increase in the interfraction time is followed by a balanced repair and killing rates.

Summing up, hyperthermia has been shown to inhibit the repair of DNA double strand breaks at temperatures $>42^{\circ}\text{C}$ ^{8, 68}. The repair kinetics model under mild hyperthermia ($<42^{\circ}\text{C}$) derived above proposes rational mechanisms of heat action on radiation induced lesions. Furthermore, this model is incorporated in the formulation of the IR and the IR cubic model to account for the effects of combining mild hyperthermia with fractionated HDRI. An enhanced response and an acceptable fit are observed after combining these 2 modalities. Moreover, repair rates estimated after the latter modality have proved the existence of a SLDR with kinetics that agree with fractionated HDRI under normal conditions.

Table 7: Results of data fitting to the IR model with its hyperthermic repair kinetics

Protocol	No Hyperther [*]	Hyperthermia
	μ (hr ⁻¹)	μ (hr ⁻¹)
1 Gy/frac	0.46 $\pm$ 0.01	0.20 $\pm$ ∞ [†]
1.5 Gy/frac	0.43 $\pm$ 0.02	0.61 $\pm$ 0.07
2 Gy/frac	0.38 $\pm$ 0.01	0.49 $\pm$ 0.12

Table 8: Results of data fitting to the IR cubic model with its hyperhthermic repair kinetics.

Protocol	No Hyperther [*]	Hyperthermia
	μ (hr ⁻¹)	μ (hr ⁻¹)
1 Gy/frac	0.41 $\pm$ 0.01	0.29 $\pm$ ∞ [†]
1.5 Gy/frac	0.38 $\pm$ 0.02	0.57 $\pm$ 0.07
2 Gy/frac	0.34 $\pm$ 0.01	0.50 $\pm$ 0.12

^{*} Results obtained from chapter 3 (figure 16)

[†] Due to the small number of data points limited by the number of possible regimens of 1 Gy dose per fraction (see section 3.3) a huge estimated error on μ is recorded

Table 9: Statistical results of the above fitting procedures

Protocol		(I R)		(I R C)	
	D.o.f.*	χ^2 **	Q	χ^2 †	Q
1 Gy/frac	12	19.05	0.09	17.94	0.15
1.5 Gy/frac	16	27.81	0.03	31.73	0.01
2 Gy/frac	16	42.20	4e-4	48.00	5e-5

* Number of degrees of freedom

** Minimum residual-square resulted from fitting the IR model

† Minimum residual-square resulted from fitting the IR Cubic model

Figure 22: Hyperthermia alone: 41°C and 43°C. The (p & c) model is used to fit the data.

Figure 23: Fractionated Hyperthermia : 43°C. The raw survival data of 2 independent experiments are shown simultaneously.

Figure 24: Fractionated irradiation combined with 15 min of 43°C hyperthermia given before every radiation fraction. Data are not normalized due to the lack of an appropriate model. HDR curve is also shown starting from the zero dose of the above treatment.

Figure 25: Fractionated HDR combined with 41°C Hyperthermia : 1 Gy/ 1 hr and 1 Gy/ 2 hr fit to IR model. HDR, LDR1 and LDR2 survival are also shown.

Figure 26: Fractionated HDR combined with 41°C Hyperthermia: 1.5 Gy per fraction at various interfraction intervals fit to IR model. HDR and LDR1 survival are also shown.

Figure 27: Fractionated HDR combined with 41°C Hyperthermia: 2 Gy per fraction at various interfraction intervals fit to IR model. HDR and LDR1 survival are also shown.

Figure 28: Repair Kinetics under Hyperthermia H: (a) 1 Gy for various interfraction intervals (b) 2 Gy for various interfraction intervals. (Derived from the IR model)

Figure 29: Repair Kinetics under Hyperthermia (H): (a) 1 Gy for various interfraction intervals. b) 2 Gy for various interfraction intervals. (Derived from the IR Cubic model)

5.0 DISCUSSION AND CONCLUSIONS

5.1 DISCUSSION

In this thesis, models for the radiation and heat action on the survival of human glioma cells were discussed. In every case, attempts were made to extend already existing models and search for reasons for inaccuracies. Furthermore, a model for combining mild hyperthermia and fractionated irradiation was derived proposing possible mechanisms under such conditions.

Three models were discussed for the case of continuous HDRI. Data fitting showed the Saturable Repair model to have the best goodness-of-fit and the smallest initial slope. The Lethal-Potentially Lethal model showed the worse fitting response and the largest initial slope. The Linear Quadratic model was in between. Furthermore, the LQ model was extended to bear 3 parameters in an attempt to enhance its fitting and behaviour at the zero dose point. This latter model, termed as Linear Quadratic Cubic model, showed the smallest initial slope among the 4 models.

Due to the reliability of its parameters, the LQ model was chosen to test for unaccounted source of error that may have played a role in 4 repeated experiments. For this purpose, a simulation experiment that considers the sources of error associated with the colony formation assay was conducted. Standard errors and correlation coefficient were estimated from a large sample of trials. The results showed

that a variation in radiosensitivity took place during the 5 month period of experiments.

In the case of radiosensitivity parameters, the mean inactivation dose showed the least variations within statistical fluctuations. These results were corroborated from the simulation experiment.

Low dose rate and fractionated HDR irradiation experiments were also performed on this cell line. Using the "incomplete repair" model which accounts for the SLD repair kinetics, an induced repair phenomenon was observed in the low dose rate up to 1 Gy dose per fraction regimen. After that, a saturation in the repair capacity was dominant. Moreover, the same results were seen when the IR model was extended to include a cubic term in the LQ model. This showed that the latter variation in the repair kinetics was not the result of an inaccurate description of continuous HDRI survival. A further possibility of a biphasic repair was ruled out due to the questionable scatter in the estimates of the slow and fast component kinetics for the various regimens.

Mild hyperthermia was applied alone and in combination with fractionated irradiation. A large degree of sensitization was observed in the case of 1 , 1.5, and 2 Gy dose per fraction regimens. In order to account for such an effect a repair kinetics model under mild hyperthermia was derived and incorporated in the IR and the IR Cubic models. The mechanisms of the latter model can be summarized as common sublethal-

nonlethal lesions present in the cell after radiation doses that can be transformed into lethal lesions under the action of heat. This model presented estimates of the repair kinetics coefficients which were found to agree with previous results under normal conditions.

In conclusion, in an entity as complex as a living cell models do describe with enough accuracy data of various treatment protocols. Understanding the mechanisms of damage and repair can be approached through the application of these various models. A saturation in the repair process as a function of radiation dose is the dominant idea. However, the incorporation of the idea of radiation induced DNA strand breaks is essential for the expression of survival probability.

Radiation alone may not be the answer for optimal tumour control in clinical settings. Mild hyperthermia in combination with LDRI treatment showed real promise⁵⁹. However, in situations where continuous LDRI treatment is followed by tumour recurrence, fractionated treatment may be the answer. Combining mild hyperthermia with fractionated irradiation proved to be successful in its outcome. Moreover, mechanisms underlying the latter protocol were proposed and supported; they are worth further investigation as well.

5.2 AREAS FOR FUTURE RESEARCH

The variation of repair rate coefficient as a function of dose per fraction warrants further investigation at the subcellular level, namely radiation single and double strand DNA breaks formation and repair. Any model describing this effect should be linked directly to radiation-DNA interaction and influence without using any adjustable parameters.

For the effect of acute hyperthermia, the extended *p&c* model (equation (109)) can not be tested *in vitro*. As mentioned before, cell grown in culture can not withstand high temperatures for long periods and stay attached to the flask. *In vivo* studies are appropriate for measuring tumour growth time as a function of heating time⁶⁴. Furthermore, modelling fractionated hyperthermia is a real challenge since repair and thermotolerance are to be included in such formulation.

Lastly, LDRI survival probability was shown to be equivalent to the extended *p&c* model of hyperthermia. Therefore, in order to account for a combined LDRI and hyperthermia treatment we double the effect of rate of induced lethal lesions in the derivation of such a model. Furthermore, fitting this model to a combined LDRI-hyperthermia data results in parameter estimation for this modality. These estimates can be compared to the ones derived from each modality separately and finally establish more confidence in the above approach.

APPENDIX

Parameter, error estimation and correlation coefficients:

Parameter estimation is done using chi-squared minimization. A minimization routine following the method of Powell²⁸ is used to fit each survival curve as a weighted least-square fit. If the vector $\mathbf{a}$ of parameter values is perturbed away from $\mathbf{a}_{(0)}$ (parameter values when X^2 is minimum), then X^2 increases. For example, in the case of one-parameter estimation if X^2 increases by 1, the region of the parameter variation will contain 68 percent of probability distribution for $\mathbf{a}$'s (corresponding to 1 standard deviation). For two-parameter estimation, the 68 percent region corresponds to an increase of 2.30 for X^2 .

Another way of estimating the probability distribution around $\mathbf{a}_{(0)}$ is to use *Monte Carlo Simulation* of synthetic data sets. If the experimenter measures data set $\mathbf{D}_{(0)}$ which gives rise to $\mathbf{a}_{(0)}$, many other realizations of the true parameters as "hypothetical data sets" can be synthesized to yield a slightly different set of fitted parameters, $\mathbf{a}_{(1)}, \mathbf{a}_{(2)}, \dots$. The assumption here is that the shape of the probability distribution $\mathbf{a}_{(1)} - \mathbf{a}_{(0)}$ is very nearly the same as the shape of the probability distribution $\mathbf{a}_{(1)} - \mathbf{a}_{\text{true}}$.

In the case of cell survival, data are generated by fluctuating the mean within the stated uncertainties back in section 2.2. The proper distribution of survival of colonies at any given dose can be proved to be Poisson. In order to

prove this, we go back to the derivation presented in section 2.2. Equation (37) states that in the absence of a sampling error the distribution of colonies is binomial. However, if we include the sampling error in the final formation of colonies, the variance of the number of colonies after propagation of error will be

$$\text{Var}(C) = S^2 \text{Var}(N) + N^2 \text{Var}(S) . \quad (113)$$

The variance of N is just N (sampling error). The variance of S is the one given in equation (37), not the final one given in equation (40) so as to avoid including the sampling error twice. Therefore, the final variance of the number of colonies is given by

$$\text{Var}(C) = S^2 \cdot (N) + N^2 \cdot \frac{S(1-S)}{N} = N \cdot S = C \quad (114)$$

Thus, the standard error of the number of surviving colonies is just the square root of that number, as for a simple Poisson process.

Poisson distribution probability is known to tend to a Gaussian distribution in the limit of a large mean. When designing an experiment, we tend to plate cells so as to get around 50 surviving colonies. For the simulation experiment, the same principle was followed. Hence, when this limit is set, it is safe to approximate the Poisson distribution by a Gaussian distribution. The Gaussian distribution of data points is crucial when using the Least Squares method so as to get robust maximum likelihood estimation.

The procedure is to draw random numbers from a Gaussian distribution. These random numbers are used to add to the considered mean a fraction of its error. After that, a data set is constructed with exactly the same numbers of measured points as actual data set $D_{(0)}$. This procedure is repeated a large number of times, each time a set of estimated parameters are derived. Finally, the Maximum Likelihood method is used to fit these sets of parameters to an M dimension Gaussian distribution probability whose central values are the initial parameters started with. The random number generator used is RAN1.

The goodness-of-fit is assessed on the basis of the value of the χ^2 -square obtained after fitting a given data set. It is defined as the area under the χ^2 -square distribution within the interval $[\chi^2, \infty]$. The χ^2 -square probability is given by

$$f(\chi^2, N) = \Gamma^{-1}(N/2) 2^{-N/2} \chi^{2(N/2-1)} e^{-\chi^2/2} \quad (115)$$

where N is the number of degrees of freedom and Γ is the gamma function. Therefore, the goodness-of-fit (Q) is the incomplete gamma function taken in the above defined limits. Strictly speaking Q is the probability that the sum of the squares of N random normal variables of unit variance (and zero mean) will be greater than χ^2 . The number of degrees of freedom is the number of points considered in the fit minus the number of parameters estimated. The Q value is estimated using the

routine GAMMQ as supplied in Numerical Recipes ²⁸ which takes care of integrating the above function.

Figure A.1 shows the histogram of the surviving fraction at 2 Gy for the 1000 repetitions. Overlay is a Gaussian distribution which has a mean=0.615 and a variance=0.008 both derived from the initial (average) parameters and assumed error. On basis of this good agreement we can trust our generation of data, especially the efficiency of producing normally distributed deviate random numbers.

Figure A.2 is a transformation of figure A.1 where now the number of surviving colonies is shown instead. Therefore, the use of the Least Square method as a maximum likelihood estimation is valid since the individual simulated data at any given dose is perfectly Gaussian distributed. Thus, we expect the minimum residual square to follow a chi-squared distribution.

The number of degrees of freedom considered in these simulations is 8. This is based on the experimental work done since usually 11 surviving fraction points per curve are considered taken in step of one Gy and 3 parameters (α , β , and the plating efficiency) for estimation in the LQ model case. Hence, the number of degrees of freedom is 11 minus 3.

Figure A.3 shows the results of binning the minimum residual squares derived from the 1000 repetitions. Overlay of the *chi-square* distribution with 8 degrees of freedom is shown which perfectly matches the binned data, except for small

statistical fluctuations which are due to the choice of binning and to the limited number of samples.

Finally, a more rigorous test of "how well we're doing" is the distribution of the goodness-of-fit. The agreement in figure A.3 can be assessed with a "*chi-by eye*" test. Figure A.5 shows the distribution of the Q values (obtained from figure A.3 by the transformation presented in figure A.4). In an ideal (infinite) situation, this distribution is uniform. If in a series of similar minimisation the Q distribution is non-uniform, then the model or the data (or both) may be flawed. If it peaks at one end (low or high end), then the measurement uncertainty is under or overestimated. In our case, within the statistical fluctuations derived from the choice of binning and the finite number of samples we feel that our Q distribution is satisfactorily uniform, and hence the distribution of parameters around the average central ones is well suited to resemble those of the true parameters.

Figure A.1: Frequency distribution of SF2. Overlay is a Gaussian distribution with the starting initial mean and assumed standard deviation.

Figure A.2: Number of colonies distribution for the 1000 simulation experiment at the 2 Gy dose. Overlay is the original Poisson distribution which is well approximated by the Gaussian distribution with a standard deviation of 7.07 (square root of 50).

Figure A.3: The distribution of the minimum residual-square for the 1000 simulation experiment. Overlay of the χ^2 distribution with 8 degrees of freedom.

Figure A.4: Q probability distribution as a function of chi-square. Note the number of degrees of freedom of every probability function.

Figure A.5: The distribution of the goodness-of-fit for the 1000 simulation experiment. The ideal situation is supposed to have a uniform distribution over $[0, 1]$.

Glossary

absorbed dose: The energy imparted to matter by ionizing radiation per unit mass of irradiated material at the place of interest. The SI unit is the gray (Gy); 1 Gy=1 joule /kg.

α/β ratio: The ratio of the parameters α and β in the Linear Quadratic model describing the shape of the survival curve or the iso-effect plot.

brachytherapy: short distance therapy where radioactive sources may be inserted into the body in direct contact with the malignant tissue. It has two forms: intracavitary and interstitial implant.

$\bar{D}$: The mean inactivation dose which is a measure of the area under the survival curve.

linear energy transfer (LET): The rate of energy loss along the track of an ionizing particle, imparted by charged particles of specified energy. Usually expressed in keV/ μ m.

potentially lethal damage (PLD): Injury that can be repaired in the radiation-free interval between irradiation and mitosis, and is lethal if not repaired.

repair half-time ($T_{1/2}$): The time required for cell repair system to repair one half of the number of lesions produced by any treatment agent.

SF2: The surviving fraction at 2 Gy derived from a survival curve model.

sublethal damage (SLD): Injury that can be repaired or will accumulate with further dose to become lethal.

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