Cancer Stem Cells, Feedback and Radiation

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Abstract

Cancer stem cells exist in tumours as a source from which mature cancer cells differentiate, analogous to healthy tissue [2]. Multiple studies have shown that xenotransplantation of human cancer initiating cells into healthy mice tissue possess the ability to regrow the cancer [5]. Radiotherapy plays a substantial role in cancer treatment; however, it has been shown that sublethal doses of radiation can induce the tumour to respawn much more aggressively, and cancer stem cells, unfortunately, are much more resistant to ionizing radiation compared to their differentiated non-stem cells [1]. Discoveries suggest that sublethal radiation can increase the rate of dedifferentiation of mature cancer cells into CSC's, causing substantial increases in the number of cancer initiating cells. We have constructed a mathematical model to investigate the dynamics of tumors, incorporating cell-cell communication and feedback among the different cell types, and the effect of ionizing radiation. We compare the model with recent experiments to determine the model parameters. To fit the data, we increased the dedifferentiation rate as the doses of radiation increased. Radiation also induced a decrease in the division rate of non-stem cells. A direct differentiation paramater was included to account for a decline in stem cell population in the experiment. The parametrized model can help suggest new treatment strategies to more effectively treat cancer patients.

1 Introduction

A mathematical model of the cell lineage that describes the interrelationship between different species in the lineage include stem cells that self renew and differentiate into multiple stem cells, committed progenitors, and terminally diffferentiated cells, along with negative feedback rates that control the self renewal rate and differentiation rates has been described by Lander et. al. We have built upon this model by accounting for dedifferentiation that occurs in our cancer

stem cell lineage model, for which there is recent evidence [1]. Dedifferentiation occurs when non-stem cells, under environmental stresses such as hypoxia or radiation, regress into stem cells. Further experimental data suggests that differentiation of cancer stem cells (CSCs) to non-CSCs may not require cell division [1]. We have incorporated this possibility into our model.

Pajonk et. al. (2012) tracked the overall progress of their induced breast cancer stem cells. We have fit our equations to their data to test the accuracy of our model. Their data was recorded through analysis of three cultures of cancer stem cells, (composed of 0%, 2%, and 10% original stem cells, respectively). Original cancer stem cells were stained red; induced cancer stem cells that arose dedifferentiation of non-stem cells were marked green. Each culture was exposed to three varying doses of radiation treatment (at increment levels of 0 Gy, 4 Gy, and 8 Gy), and the number of induced stem cells that proliferated between each treatment cycle was recorded. The parameter v_0, which we will later use, is the proliferation rate at which stem cells (original and induced) divide and self-renew to give rise to identical stem cells or differentiate to yield non stem cells. This is more commonly known as: "differentiation with division". We also later represent the division of non-stem cells with the parameter v_1. From the experiment, we have these values fixed to be, $v_0 = \frac{2}{7} days^{-1}$, and $v_1 = \frac{5}{7} days^{-1}$ [2].

Another mathematical model we are drawing upon is the linear-quadratic (LQ) model, which quantifies the effects of radiation upon cancer stem cells by calculating the fraction of cells that survive a certain dose after damage to DNA [4].

Pajonk et. al. (2012) has investigated the pathways that regulate the reprogramming of differentiated cells into stem cells [1]. His experiments and data are the framework of our model dynamics and equation design. They tracked the CD24[low]/CD44[high] cancer stem cells as they were targeted with radiation, and analyzed them via flow cytometry. They concluded that the radiation-targeted cancer stem cells exhibited an increase in population due to dedifferentiation [2]. Dedifferentiation also occurs in normal tissues as well: they observed induced stem cells under 0Gy control group; thus, dedifferentiation had occurred [1]. In addition, they have developed ways to track the cells more effectively by irradiating cancer stem cells in vivo in mice and stained for ZSGreen fluorescent protein activity in vitro to determine the tumorigenicity of these cells [5]. The expression of ZSGreen is normally eliminated by the 26S proteasome in healthy cells. However, inactivation or decreased activity of the 26S proteasome leads to increased concentrations of ZSGreen protein aggregates, which is commonly seen in breast cancer stem cells. They have determined that

the irradiated cancer stem cells that expressed the ZSGreen fluorescent protein markers exhibited CSC-like properties, such as increased rate of division and CSC markers [5], and thus can test for the activity of the 26S proteasome.

Fractionated radiotherapy has been proven to increase the dedifferentiation rate in cancer cells. However, as a result of this increased dedifferentiation rate, proliferation rates decrease for non-stem cells [1]. Radiotherapy has been noted to induce mature cancer cells to exhibit certain cancer stem cell markers, such as CD24[low]/CD44[high] [2]. This marker system means that certain cells with low concentrations of CD24 marker on the cell membrane surface and high concentrations of CD44 marker on the surface exhibit tumorigenic properties, the functioning definition of "cancer stem cells". Why sublethal radiation causes higher rates of dedifferentiation is not well understood. It is believed that radiation activates certain stem cell activating genes that exhibit pluripotent properties. These (in a normal stem cell scenario) are called induced pluripotent stem cells [2] which have been investigated as a possible alternative to embryonic stem cells. Another finding that can account for the increased population in cancer stem cells in response to radiation is that under fractionated radiation therapy, cancer initiating cells were spurred from a quiescent G0 phase into active cell cycling phase, where they quickly proliferate [2].

2 Math Model

We construct a mathematical and computational model to investigate the population dynamics of the three cell types from the experiment (original, induced, and non-stem) and to describe the experimental data using a minimal number of parameters. Stem cells self-renew and defferentiate through asymmetric and symmetric divsion. Stem cells may also directly differentiate (differentiation without division); they move onto the next cell type in the lineage without replicating. Non-stem cells are comprised of stem cells that progress down the cell lineage, which is the fraction of stem cells that do not self-renew. Non-stem cells can die off, or dedifferentiate into a less differentiated stage. Figure 1 depicts a schematic of lineage model that includes feedbacks.

Original, induced, and non-stem cells self-renew and divide at certain rates. The p parameters represent self-renewal fraction, while the v parameters represent mitosis rates. $\overline{d_o}$ and $\overline{d}_i$ describe the direct differentiation rates of original and induce stem cells, respectively. λ is the rate of dedifferentiation, while d is the death rate of non-stem cells. Negative feedback is applied on the self-renewal parameters and dedifferentiation parameter to account for a communication and feedback

among the cells.

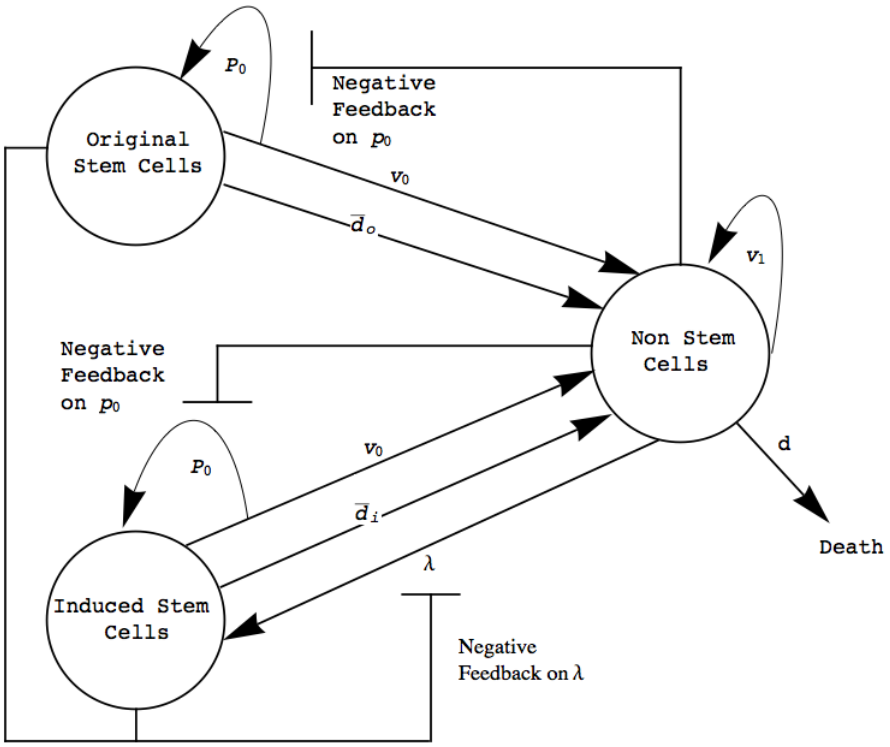

Figure 1: Depiction of a cell lineage with three cell types.

(1) $x'_{os}(t) = (2p_0 - 1)v_0 x_{os}(t) - \bar{d}_o x_{os}(t)$

(2) $x'_{is}(t) = (2p_0 - 1)v_0 x_{is}(t) + \lambda x_{ns}(t) - \bar{d}_i x_{is}(t)$

(3) $x'_{ns}(t) = 2(1 - p_0)v_0(x_{os}(t) + x_{is}(t)) + \bar{d}_0 x_{os}(t) + \bar{d}_i x_{is}(t) + (v_1 - d - \lambda)x_{ns}(t)$

The system of equations represents the change in population of cells over time, t. We use this system to model the scenerio depicted in Figure 1. We assume that the original and induced stem cells have similar properties; they self-renew, differentiate, and directly differentiate. Induced stem cells are acquired through dedifferentiation, which we track separately from original stem cells as in the experiments ([2] and [1]). Although progenitors and terminally differentiated cells belong to the non-stem population, we do not distinguish them since the experimental data does not distinguish between these cell types ([2] and [1]) . Equations (1-3) demonstrate the system of ordinary differential equations we use to model the scenario presented in Figure 1.

Since original and induced stem cells hold similar properties, we decide to have them with equal division rates. For the self renewal fraction, we decided to have two cases where p_0 was the same for equation (1-2) and a case where equation 2 is introduced with a new self renewal fraction, p_1, where p_1 becomes the self-renewal rate for induced stem cells. $p_1 will be further explained in section 3.2.1$. Since division produces two cells, we multiply v_0 to the portion of stem cells that stay in the same lineage, p_0. $-v_0$ is used to show the loss of a stem cell when the system undergoes symmetric differentiation. The $2(1-p_0)$ expressions found in the equation for non-stem population represents the portion that progress through the lineage. The v-parameters are the mitosis rates. Progenitors replicate and differentiate, so for simplicity, we choose to model these two phonomena with one parameter,v_1. The death rate, d, comes from the terminally differentiated cells belonging to the non-stem population. Table 1 lists and describes the variables and parameters of the system of differential equations.

x_{os}	Original Stem Cell Population
x_{is}	Induced Stem Cell Population
x_{ns}	Non Stem Cell Population
p_0	Portion of Stem Cells that stay in the same lineage
v_0	Mitosis (division) rate of Stem Cells
v_1	Mitosis (division) rate of Non-Stem Cells
λ	Dedifferentiation Rate
$\bar{d_o}$	Direct Differentiation without division from Stem to Non Stem Cells
$\bar{d_i}$	Direct Differentiation without division from Induced Stem to Non Stem Cells
d	death rate of Non Stem Cells

Table 1: Parameters

3 Steady State Analysis

We begin by analyzing the behavior of the lineage model. In particular, we ask under what conditions does the model support the existence of steady-states. Steady states are important biologically as they imply that the model can describe homeostasis. Having a model that converges to zero or diverges off to infinity past the time we focus on, is not an accurate depiction of how cells work in the body. For this, we conduct analysis on our model with various conditions on parameters to find the best representation.

3.1 Population Dynamics with Constant Parameters

We first consider the simple case when all parameters are considered constant in equations (1-3) to minimize the number of unknown parameters. In particular, we set the feedback gains to zero. Setting the right hand side of the equations (1-3) equal to zero, we conclude a steady state with the following conditions. From equation 1, we can conclude,

$$(4) \qquad p_0 = \frac{1}{2}(\frac{\bar{d_o}}{v_0} + 1)$$

The above equation for p_0 shows that as long as this equation is satisfied a steady state will occur for x_{os} since p_0,v_0, and $\bar{d_o}$ are the only parameters that x_{os} depends on. From equation 2, we solve for x_{ns} and find

$$(5) \qquad x_{ns} = \frac{\bar{d_i} - \bar{d_o}}{\lambda} x_{is}$$

The equation above raises the conditions that need to be satisfied. In order for x_{ns} to be a positive number, $\bar{d_i} > \bar{d_0}$ and $\lambda > 0$. In order for x_{ns} to reach a steady state, the rate of direct differentiation of induced stem cells needs to be greater than that of the rate of direct differentiation by original stem cells. Also, dedifferentiation needs to occur to produce a steady state. From equation 3, we solved for x_{is}:

$$(6) \qquad x_{is} = \frac{(2(1-p_0)v_0 + \bar{d_o})\lambda x_{os}}{2(p_0-1)v_0\lambda - \bar{d_o}\lambda + d - v_1}$$

In order to keep $x_{is} > 0$, more conditions need to be satisfied. The numerator will always be positive, therefore the problem arises in the denominator. In order to reach a steady state,
$2(p_0-1)v_0\lambda - \bar{d_o}\lambda + d - v_1 > 0$. When all these conditions are satisfied, a steady-state solution of the model exists.

Although this mathematical model has steady state solutions, these solutions are very special. In particular, the stem cell population is identically constant and equal to its initial condition. In real biological systems, the numbers of cells may vary with the environmental conditions and the number of progeny. Therefore, we came to the conclusion that there needed to be a feedback factor on p_0 to avoid the constraints for the steady state condition of the system.

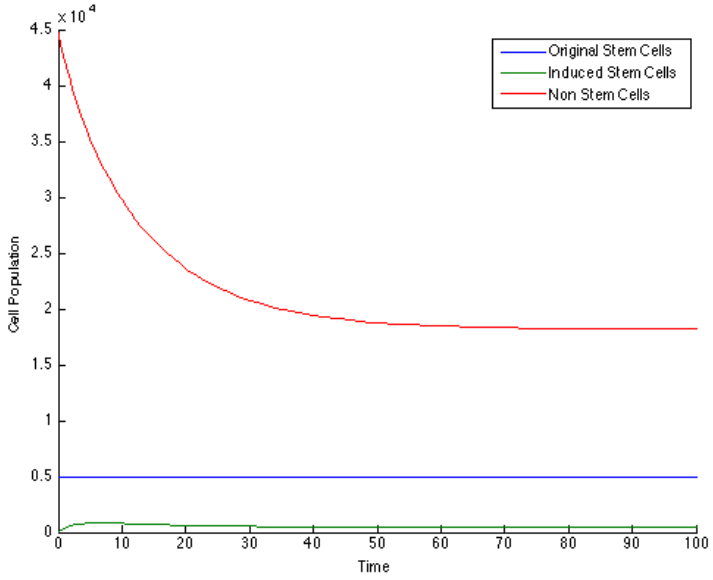

Figure 2: The graph above depicts the steady state of original stem cells, induced stem cells, and non stem cells. As predicted from the steady state analysis, x_{os} is constantly at steady state and equal to its initial value. x_{is} eventually reaches a steady state around $t = 35$ after some fluctation. x_{ns} decreases dramatically from $0 < t < 30$ and then reaches a steady state around $t = 70$. The values of the parameters used are given in Table 2.

p_0	λ	$\bar{d}_o$	$\bar{d}_i$	d
0.5	0.01	0	0.4	0.8

Table 2: These are the parameters that are used in figure 2. d_o was chosen to be zero in order for the original stem cell population to be constant and reach a steady state as seen from the condition from equation 4.

3.2 Population Dynamics and the Effects of Feedback

Cells may communicate with each other through feedback regulation of intercellular processes such as self-renewal probabilities of cells and cell division rates. Feedback may be positive, which results in an upregulation of cellular processes or negative, which downregulates cellular processes. Feedback may occur over long ranges, mediated by diffusible chemical factors, or occur over short-ranges mediated by cell-cell contact. We do not distinguish this difference here as we are

using a spatially homogeneous model based on ordinary differential equations.

3.2.1 Self-Renewal Fraction Parameter on Population Dynamics

We first investigate the effect of negative feedback on the self-renewal probability of stem cells where:

$$(7) \qquad p_0 = \frac{\overline{p}_0}{1 + g_0 x_{ns}}$$

here g_0 is a feedback gain factor representing sensitivity to feedback. Biologically, this type of negative feedback is achieved by members of the TGFβ superfamily of proteins [3]. Our steady state solutions for our system of equations now changes. By solving for x_{ns} from (1) we get,

$$(8) \qquad x_{ns} = \frac{v_0(2\overline{p}_0 - 1) - \overline{d}}{g_0(\overline{d} + v_0)}$$

Clearly, this introduces the additional condtion of $\overline{p}_0 > \frac{1}{2}$ and $v_0(2\overline{p}_0 - 1) > \overline{d}$ to obtain biologically relevant solutions. In other words, more than half of original stem cells must replicate, and direct differentiation should occur at a pace slower than differentiation with division. If $p_0 < 1/2$, then the dynamics shows that the original and non-stem populations decay to zero in time. We also notice that g_0 has to be small enough to have a large non-stem population, as we have in the experiment. From equation (2), we obtain the same conditions on $\overline{d}_i$ and $\overline{d}$ acquired from the constant parameter study. From equation (3) we obtain

$$(9) \qquad v_0 x_{os} + (v_0 + \overline{d}_i - \overline{d}) x_{is} = (\lambda + d - v_1) x_{ns}$$

Now the condition placed on us is $\lambda + d > v_1$. Thus, increasing the death rate d implies that the dedifferentiation rate should decrease, and vise-versa, in order for the system to have a nontrivial steady state. So death and dedifferentiation of non-stem cells are proportional to each other. From a biological perspective,this makes sense; if the target cells are dying off quickly, this should not increase a desire for dedifferentiation, or else the non-stem cell population would vanish. Also, if non-stem cells are rarely dying off, then the dedifferentiation rate is basically 0, and non-stem cells grow exponentially. Although our numerical solution gives us the steady state we're looking for (Figure 7), it does not fit the experiment.

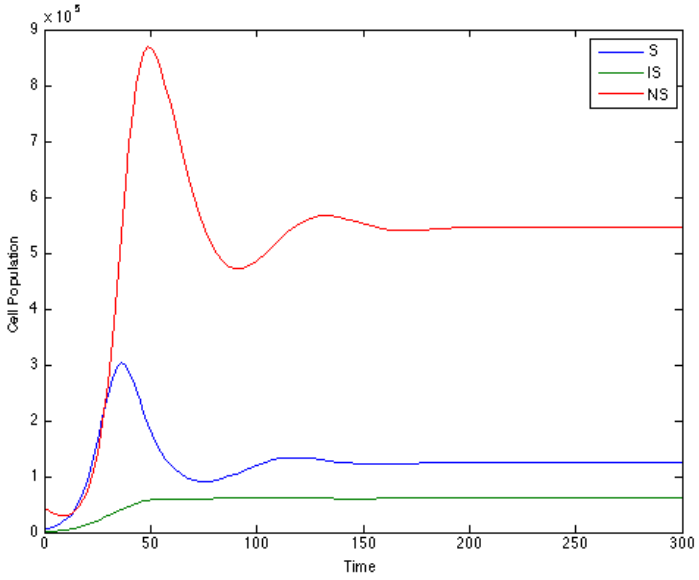

Figure 3: Steady State solution with negative feedback on self-renewal of original stem cells. The initial decrease in non-stem cells is caused by $\lambda + d > v_1$, which decreases the population, and the increase in original stem cells. Since non-stem also depends on original and induced stem cells, the increase both these populations boost the number of non-stem cells dramatically.

$\bar{p_0}$	λ	$\bar{d_o}$	$\bar{d_i}$	d	g_0
0.8	0.01	0.01	0.1	0.8	10^{-7}

Table 3: These parameters were used to reach a steady state with feedback on p_0 as showed on figure 3. Compared with table 2, p_0 has more freedom because it does not depend on d_o and v_0. As for d_o, it has increased from the case with all constant parameters.

3.2.2 Self-Renewal and Dedifferentiation Parameters on Population Dynamics

Next, we investigate the effect of negative feedback on both p_0 and λ, the dedifferentiation rate. The negative feedback on λ is regulated by the stem cells. We believed that such feedback would limit the growth of x_{ns} because dedifferentiation would need to occur more often. The feedback on p_0 is the same as from section 4.2.1. The parameter λ is

now given by:

$$\lambda = \frac{\bar{\lambda}}{1 + g_1(x_{os} + x_{is})} \tag{10}$$

This new λ replaces all the existing λ's and is now used in equations (2) and (3). Like p_0, λ also has a feedback gain (g_1) that regulates the sensitivity to feedback. We applied steady state analysis to observe the behavior of feedback on these two parameters. Setting the right hand side of equations (1-3) equal to zero, we were able to do so. From equation 1, we observe the steady state conditions for x_{ns}.

$$x_{ns} = \frac{1}{g_0}(\frac{2p_0v_0}{\bar{d_o} + v_0} - 1) \tag{11}$$

We realized g_0 needed to be a very small to reach a realistic number of non stem cells. Also, this leads to the condition $\frac{2p_0v_0}{d_o+v_0} > 1$ because we require $x_{ns} > 0$. From equations (2) and (3) we obtain the steady-state OSC and ISC populations:

$$x_{os} = \frac{1}{g_1}(\frac{\bar{\lambda}x_{ns}}{(\bar{d_i} - \bar{d_o})x_{is}} - g_1x_{is} - 1) \tag{12}$$

$$x_{is} = \frac{\bar{\lambda}x_{ns}}{(\bar{d_i} - \bar{d_o})g_1(\frac{x_{ns}(d-v_1)}{v_0} + \frac{1}{g_1})} \tag{13}$$

It appears more conditions arise when trying to reach a steady state. The condition $\bar{d_i} > \bar{d_o}$ appears once again. Along with this reoccuring condition, $d > v_1 = \frac{5}{7}$ and $\frac{\bar{\lambda}x_{ns}}{(\bar{d_i}-\bar{d_o})x_{is}} - g_1x_{is} > 1$. With these conditions met, a steady state will be reached.

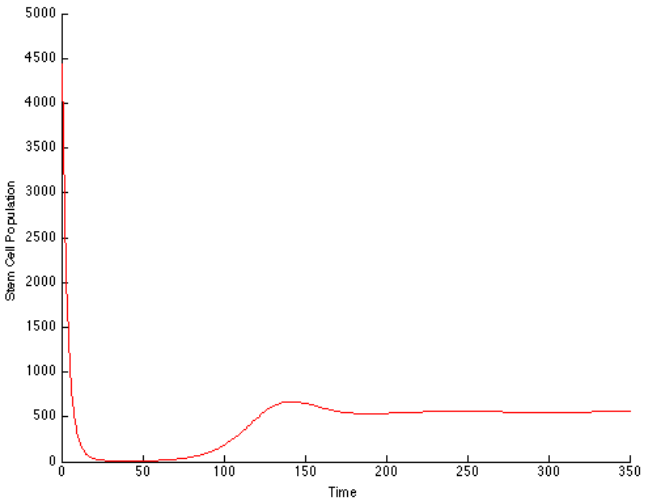

Figure 4: This graph depicts how the original stem cell population behaves with negative feedback on λ and p_0 as it reaches a steady state. As seen above, the stem cell population decreases dramatically to the point of almost extinction but soon after reaches a steady state.

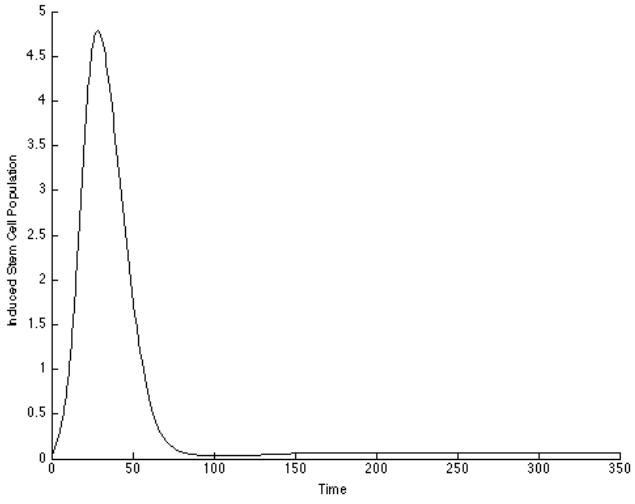

Figure 5: This graph depicts the behavior of induced stem cells with negative feedback on λ and p_0. The induced stem cell population increases only a few cells and then decreases to a steady state.

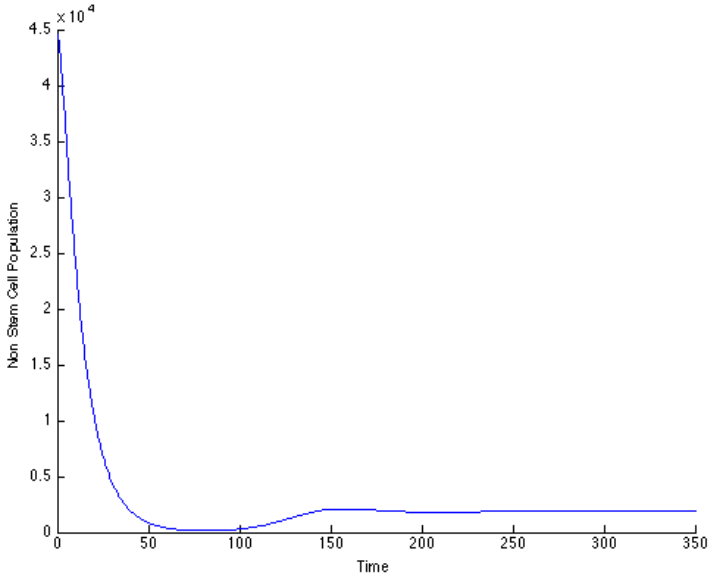

Figure 6: The population of non stem cells behave similar to that of the original stem cells with negative feedback on λ and p_0. The non stem cell population decreases at a very fast rate and then reaches a steady state.

$\bar{p_0}$	λ	$\bar{d_o}$	$\bar{d_i}$	d	g_0	g_1
0.8	0.01	0.1	0.6	0.8	10^{-5}	1

Table 4: These were the values that were used to reach a steady state with feedback on p_0 and λ (Figure 4 -6). Many parameters stayed the same from table 3, but $\bar{d_i}$ increases dramatically to keep the induced stem cell population from growing.

3.2.3 Self-Renewal Fraction Parameter for Induce Stem Cells

We next relax the condition that original and induced stem cells share the same self-renewal rate to better control the induced stem population. We do this by replacing the p_0 coefficients of x_{is} with p_1 in the system of equations found in Equations (1-3). We apply negative feedback on this new parameter, and give it its own gain factor. Therefore,

$$p_1 = \frac{\overline{p}_1}{1 + g_2 x_{ns}} \tag{14}$$

The expression we previously obtained for x_{ns} from the first equation in equations (1-3) holds here. At steady state, we get two expressions from equations (2) and (3) respectively:

$$(15) \quad \lambda(1 + g_2 x_{ns})x_{ns} = ((1 + g_2 x_{ns} - 2\overline{p}_1)v_0 + \overline{d}_i)x_{is}$$

$$(16) \quad v_0 x_{os} + (2v_0(\frac{1 + g_2 x_{ns} - \overline{p}_0}{1 + g_2 x_{ns}} + \overline{d}_i)x_{is} = (\lambda + d - v_1)x_{ns}$$

We can see from equation (15) $1 + g_2 x_{ns} > 2\overline{p}_1$, this is true if $\bar{p}_1 <$ 1, which it must be. This also carries over onto the relationship we get from (3). A small g_2 and $\overline{d}_i$ coupled with $\overline{p}_1 > \frac{1}{2}$ could produce an unstable solution to the system, however we are not interested in this since it would result in an exponential growth of induced stem cells. The result we get from (3) gives us the same conditions on dedifferentiation and death rate that equation (3) gave in the previous parameter study. Overall, introducing p_1 with negative feedback only slightly changes the population of induced stem cells, as seen in Figure 7, but it may prove useful with further study.

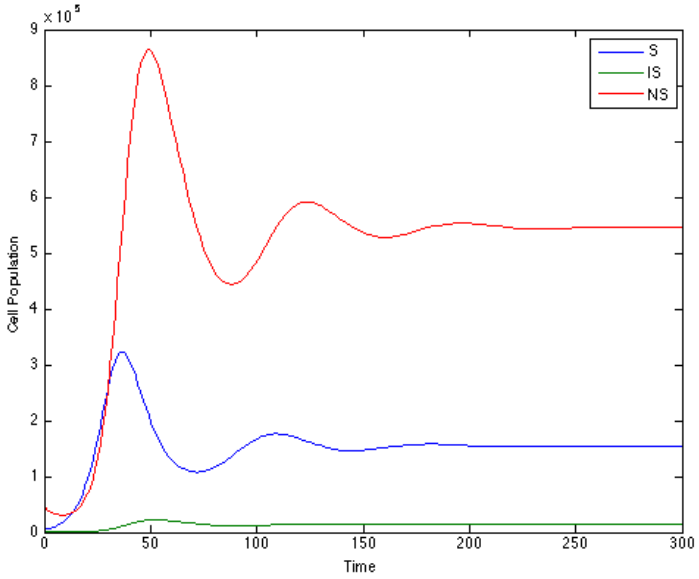

Figure 7: Steady state solution with negative feedback on self-renewal paramaters for original stem and induced stem cells,p_0 and p_1 respectively. There is a slight decrease in induced stem cell population compared to Figure 3. No significant change from having the same self-renewal rate for original and induced stem cells.

$\bar{p_0}$	$\bar{p_1}$	$\bar{\lambda}$	$\bar{d_o}$	$\bar{d_i}$	d	g_0	g_1	g_2
0.8	0.8	0.01	0.01	0.1	0.8	10^{-7}	0	1

Table 5: Above are the values that were used to reach a steady state with feedback on p_0 and p_1 for figure 7. The values used in table 4 have been modified to reach a steady state for this condition. Comparing the values from table 4, we can obseve that the gain for lambda is turned off and the parameter for direct differentiation for original stem cells has decreased even more.

3.2.4 Logistic growth for mitosis

The experiment we are attempting to model has its population of cells growing in subculture wells, hence our model is subject to a carrying capacity. We implement a modified logistic growth on the division rates to cap the total population of all three cell types. Without modified logistic growth, we are not able to match the experimental data from [2] [1]. $a = 1$, gives a total population that is too low. Hence, we take the cell division rates:

$$v_0 = \overline{v}_0(1 - (\frac{x_{os} + x_{is} + x_{ns}}{k})^a) \tag{17}$$

$$v_1 = \overline{v}_1(1 - (\frac{x_{os} + x_{is} + x_{ns}}{k})^a) \tag{18}$$

where k is the carrying capacity, and $a = 1$ gives us regular logistic growth. By increasing a we can reduce the rate of cell death when numerically solving the system of equations. From a steady state analysis, equation(1) gives

$$x_{os} + x_{is} + x_{ns} = k(1 - \frac{\overline{d_o}}{(2p_0 - 1)\overline{v}_0}) \tag{19}$$

Which tells us that $1 > \frac{\overline{d_o}}{(2p_0-1)\overline{v}_0}$, which implies $\overline{d_o} < (2p_0-1)\bar{v_0}$ (same as before). This also implies that $p_0 > \frac{1}{2}$ Applying feedback, we see that $x_{ns} < \frac{2\overline{p}_0-1}{g_0}$, and again we have $\overline{p}_0 > \frac{1}{2}$. From equation(2),

$$x_{ns} = \frac{1}{\lambda}(\overline{d}_i - \frac{\overline{d_o}(2p_1 - 1)}{2p_0 - 1})x_{is} \tag{20}$$

A simple way to reach a positive value for the coefficients would be to set $p_1 < \frac{1}{2}$. If this is not the case, then $(2p_0 - 1)\overline{d}_i > (2p_1 - 1)\overline{d_o}$, a condition that can be met with a large enough value for the rate of direct differentiation of induced stem cells. If original and induced stem cells directly differentiate at the same rate, then original stem cells

must replicate faster than the induced stem cells. From equation(3), we get a simlar condition as in the previous cases, that is

$$d + \lambda > \frac{\overline{v}_1 \overline{d_o}}{\overline{v}_0(2p_0 - 1)} \quad (21)$$

We see that the rate of non-stem cells being lost due to cell death and dedifferentiation should be high, compared to its division rate.

4 Cell Population Dynamics with Varying Doses of Radiation

The experimental data that we reference below comes from Pajonk's âĂIJthree culturesâĂİ experiment described above, involving cultures with 0%, 2%, and 10% stem cell composition from an initial population of 50,000. Each culture was exposed to 0 Gy, 4 Gy and 8 Gy of ionizing radiation, and the number of original and induced stem cells, the standard deviation values, and total population/standard deviation were recorded.

Initial Orig. Stem Cells	Orig. Stem Cells	Orig . Std. Dev.	Induced Stem Cells	Induced Std. Dev.	Total Population	Total Std. Dev.
0%	0	0	206.8	31.1	425168.9	11470.8
2%	95.5	39.5	197.6	20.3	425168.9	11470.8
10%	632.5	228.4	134.5	25.5	425168.9	11470.8

Table 6: This data table represents the population dynamics of stem cells, induced stem cells, and non stem cells without radiation in a seven day time span. The initial cell count was at 50,000 for three different initial original stem cell percentages.

Initial Orig. Stem Cells	Orig. Stem Cells	Orig . Std. Dev.	Induced Stem Cells	Induced Std. Dev.	Total Population	Total Std. Dev.
0%	0	0	658.2	30.9	263106.3	8477
2%	147	7	470.9	140.7	263106.3	8477
10%	970	70.7	349.3	35.2	263106.3	8477

Table 7: This data table represents the population dynamics of original stem cells, induced stem cells, and non stem cells in a seven day time span. This cell culture was exposed to 4 Gy radiation on day 1 of the experiment. The initial cell count was at 50,000 for three different initial original stem cell percentages.

Initial Orig. Stem Cells	Orig. Stem Cells	Orig . Std. Dev.	Induced Stem Cells	Induced Std. Dev.	Total Population	Total Std. Dev.
0%	0	0	2133.6	495	160781.6	4849.8
2%	177	75	1418.2	470.3	160781.6	4849.8
10%	1088	39.6	847.7	402.7	160781.6	4849.8

Table 8: This data table represents the population dynamics of stem cells, induced stem cells, and non stem cells in a seven day time span. This cell culture was exposed to 8 Gy radiation on day 1 of the experiment. The initial cell count was at 50,000 for three different initial original stem cell percentages.

4.1 The effects of 0 Gy Radiation

We attempt to match the experimental data in the case without radiation using our model. In the system of equations, we have orginal and induced stem cells have their own self-renewal rates(p_0 and p_1) with feedback. We also apply feedback on the dedifferentiation rate, λ, and impose a modified logistic growth on division rates. After experimentations, we were able to determine parameters that yielded a good fit between the model and experimental results, however, the original stem cell population would tend to zero, while the other two populations converged. In order to reach a steady state for all the cell types, we apply modified logistic growth on the direct differentiation parameter for original stem cells, $\bar{d_o}$.

$$\bar{d_o} \equiv \bar{d_o}(1 - \frac{x_{os} + x_{is} + x_{ns}}{k}) \tag{22}$$

The given carrying capacity is 425,000 cells, as suggested by the experimental results in Table 6. The model predicts that the populations reach a steady state within the standard deviations for the experimental data for all cases considered– 0%, 2% and 10% original stem cells. Initial stem cell data, as shown in Figures 8-10 and Tables 9-12.

Feedback on the self-renewal rates of original stem cells keeps them from self-renewing (and dividing) rapidly in the presence of a large nonstem cell population. This feedback also diminishes the effect of the self-renewal paramater because of negative feedback. Both direct differentiation rates for original and induced seem to be at values that steadily contribute to the non-stem cell population. Logistic growth on $\bar{d}$ ensures that not all stem cells directly differentiate, while $\bar{\lambda}$ competes with $\bar{d_i}$. The number of induced stem cells increase because even though $\bar{\lambda}$ is small, a portion of the large number of non stem cells is still able to dedifferentiate. In this case, where no radiation is applied, the dedifferentiation parameter $\bar{\lambda}$, is quite low. An overwhelming presence of non-stem cells does not seem to excite dedifferentiation; radiation,

however, doses we see in the next section.

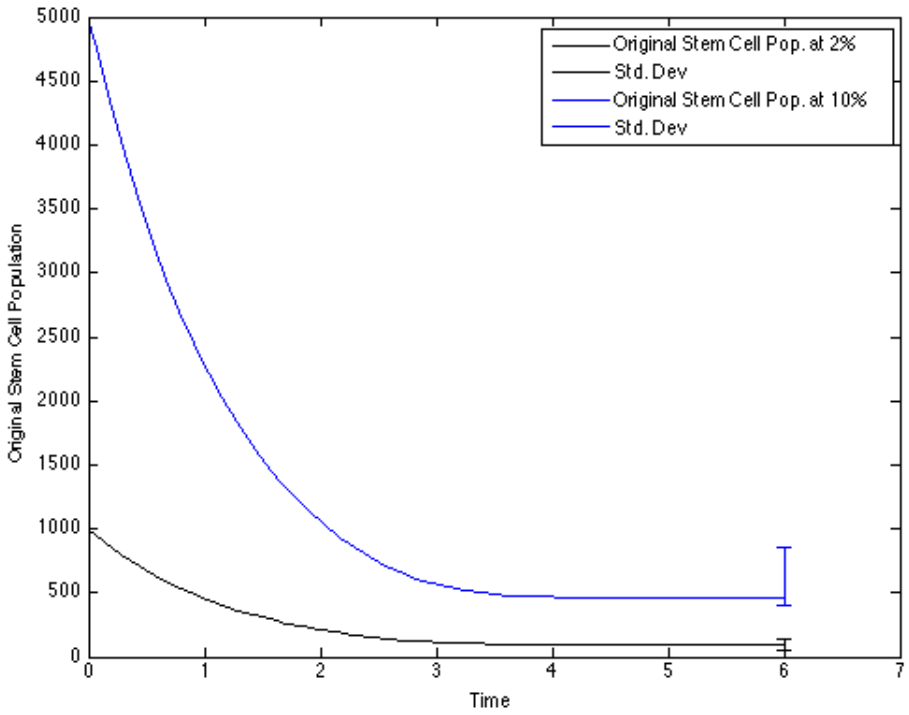

Figure 8: This set of graphs shows the population dynamics of original stem cells with no radiation treatment. When no stem cells are present at time 0 (e.g., no original stem cells) there are no original stem cells at any later time. For the cases of 2% and 10% original stem cells, there is a gradual decrease in the span of six days but eventually the population reaches a steady state.

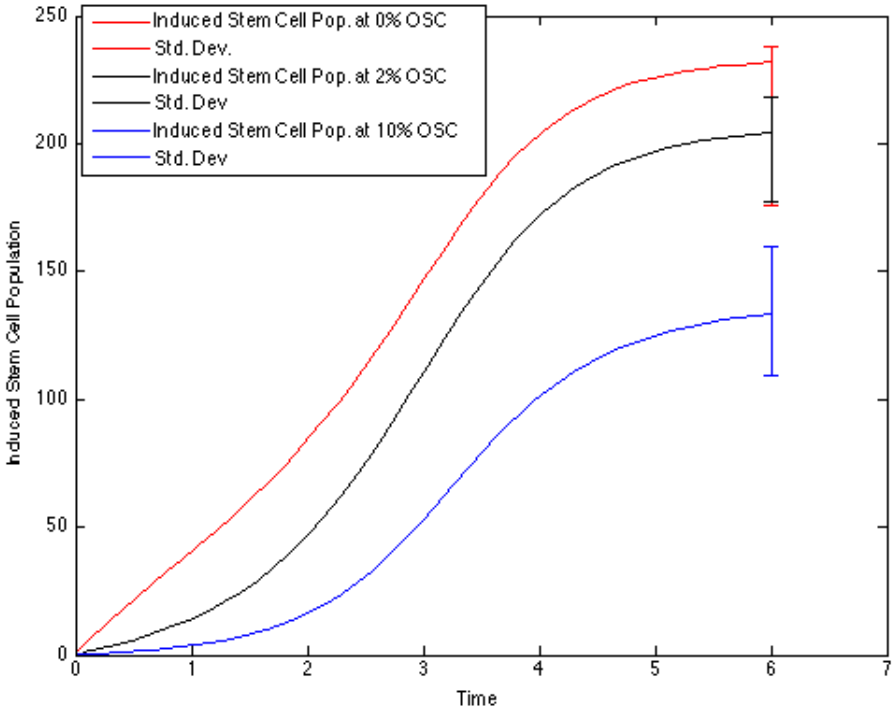

Figure 9: This set of graphs shows the induced stem cell population dynamics with 0%, 2% and 10% of original stem cells with no radiation. The initial population size for all three cases of induced stem cell start at zero. All the induced stem cell populations increase with a logistic growth and reach a steady state.

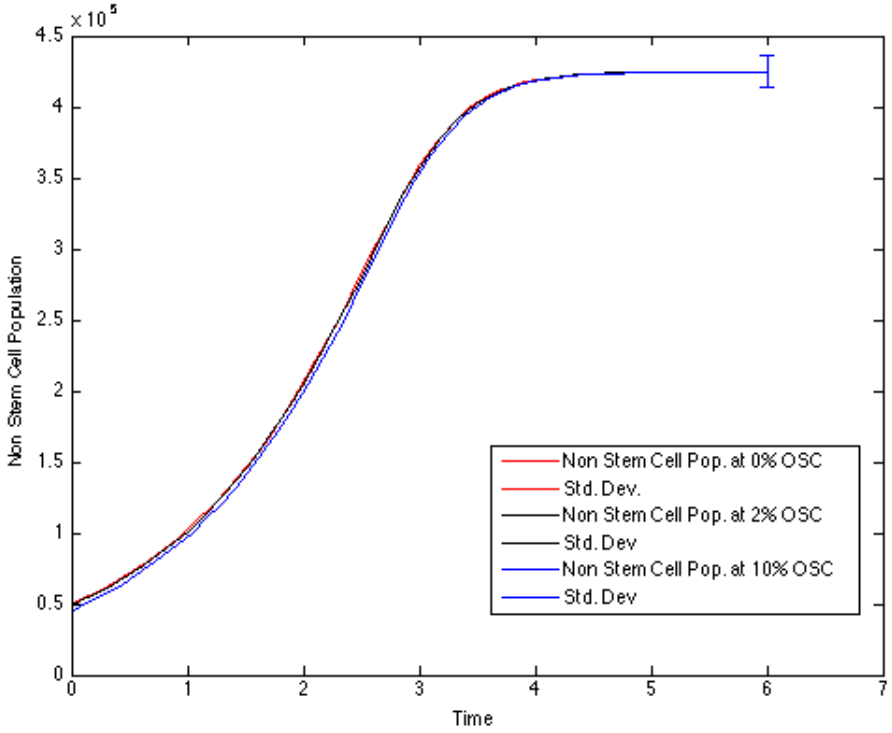

Figure 10: This set of graphs shows the non stem cell population dynamics with 0%, 2% and 10% of original stem cells. These populations all increase and have characteristics of a logistic curve with a steady state.

$\bar{p_0}$	$\bar{p_1}$	$\bar{\lambda}$	d_o	$\bar{d_i}$	d	g_0	g_1	g_2	a
1	0.1	0.001	0.5	0.9991	0	1	.0035	1	4

Table 9: The parameters above were used to successfully match the experimental data as shown in figures 8-10.

	Numerical	Experimental	Std. Deviation
x_{os}	0	0	0
x_{is}	231.9	206.8	31.1
Total Population	424744.9	425168.9	11470

Table 10: This table represents the values at day 6 for the case where there are no original stem cells.

	Numerical	Experimental	Std. Deviation
x_{os}	94.5	95.5	39.5
x_{is}	204.3	197.6	20.3
Total Population	424677.5	425168.9	11470

Table 11: This table represents the values at day 6 for the case where the initial population consists of 2% SCs and 98% non-stem cells.

	Numerical	Experimental	Std. Deviation
x_{os}	460.3	632.5	228.4
x_{is}	133.5	134.5	25.5
Total Population	424380.7	425168.9	11470

Table 12: This table represents the values at day 6 for the case where the initial population consists of 10% stem cells and 90% non-stem cells.

4.2 The Introduction of Radiation

In order to model radiation with our system, the LQ model described above was applied. In the experiment from [2] [1], radiation is applied once on day 1. Then the cell populations are quantified 5 days later on day 6. Therefore, we applied the LQ model [4] to the cell populations on day 1, which results in a discontinuity of the populations in time. The original stem cells were assumed to be less susceptible to radiation [1], and to simplify the model, we assumed that the original and induced stem cells were insensitive to radiation treatment and so their surviving fraction is one. The surviving fraction of non stem cells from the LQ model is given by

$$S = e^{-dose(\alpha+\beta dose)} \tag{23}$$

This equation was derived as a solution from a system of equations [4]. In this model, S represents the fraction of cells that survive radiation dose. *dose* represents the radiation dose intensitiy(in Gy) and α and β represent the cell sensitivity to radiation, quantified by the number of double strand breaks (DSB's) in the cell. For the following cases involving radiation, we are setting $\alpha = 0.06606$ and $\beta = 0.01352$ to find the surving fraction of non stem cells as seen on equation (23).

4.2.1 The Effects of 4 Gy

We use the same parameters as we did for the 0Gy radiation case. Now, once radiation is applied (on day 1), we apply the LQ model to get the number of surviving cells, and use this fraction as initial conditions to

solve the sytem from the first day to the sixth. In order to match the experimental observations, we also see that the dedifferentiation rate has to increase by 5 times the initial amount. After radiation occurs, we also decrease the rate of division for non-stem cells, as suggested by Lagadec [2].

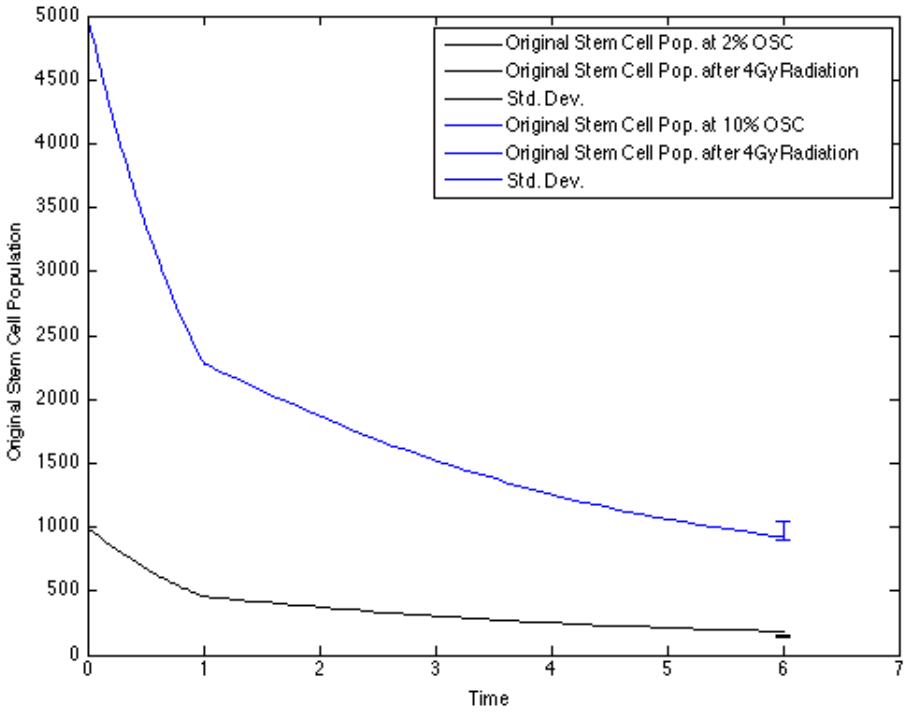

Figure 11: This set of graphs shows the population dynamics of original stem cells with 4 Gy radiation. With 0% original stem cells, there is no increase of population. When there are 2% stem cells present initially, the number of original stem cells decreases significantly over time, and the rate of decrease slows after radiation is applied. Note the kink in the curve at day 1, which is due to radiation-induced death of non stem cells. As you can see from the error bar and tables 14-16, we were not able to match the original stem cell population at day 6 for 2%.

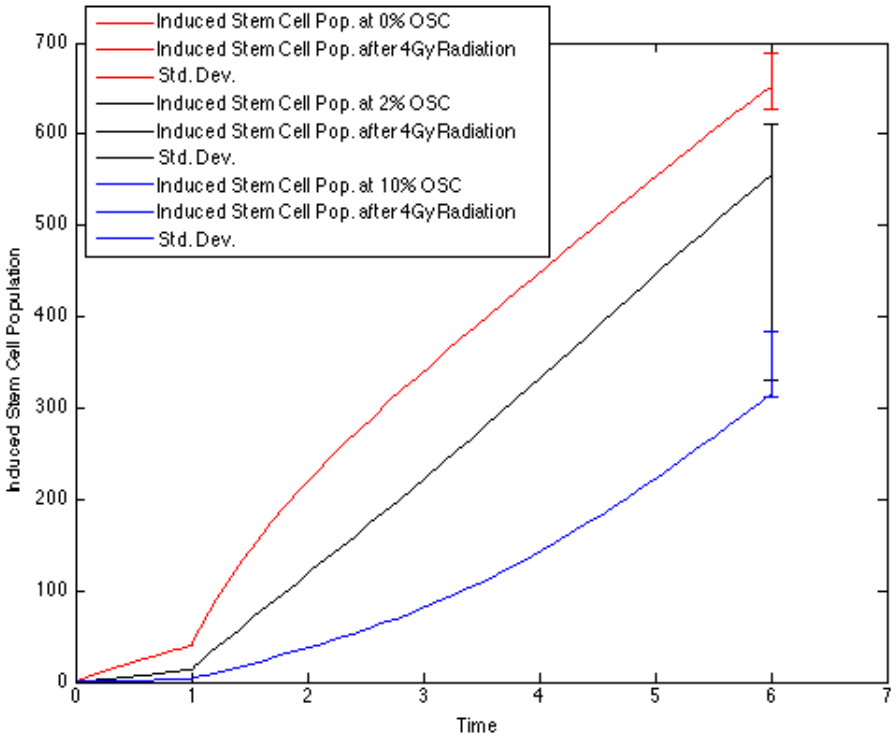

Figure 12: This set of graphs shows the induced stem cell population dynamics with 0%, 2% and 10% of original stem cells with 4 Gy radiation. The initial population size for all three cases of induced stem cell start at zero. Similar to that of original stem cell population, induced stem cell population also has a slight kink at day 1 from the radiation applied.

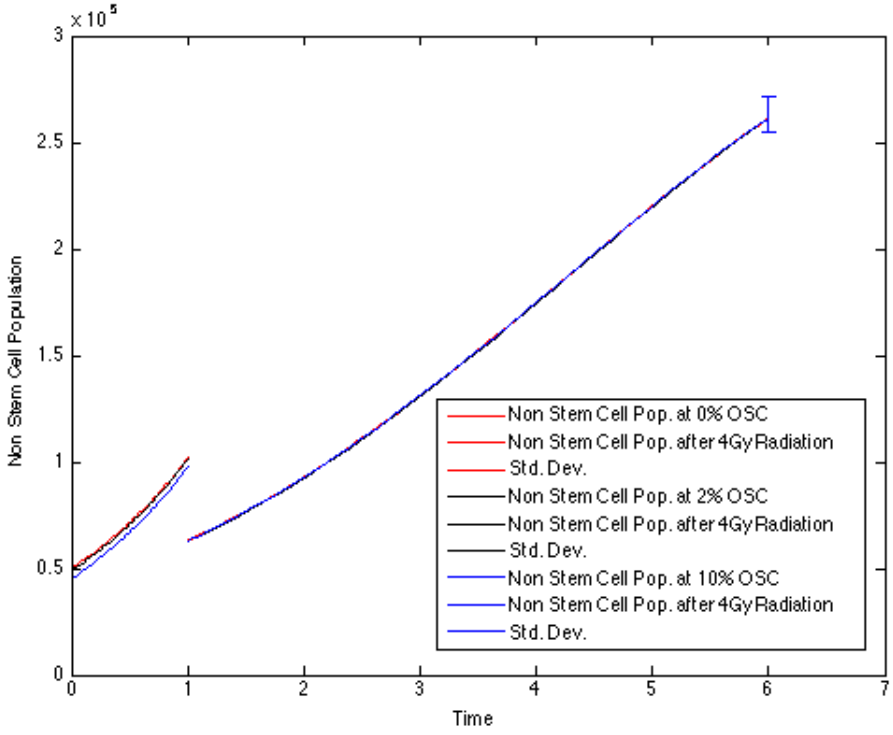

Figure 13: This set of graphs represents the non stem cell population dynamics with 0%, 2% and 10% of original stem cells. This set of graphs form a form a discontinuity from the radiation-induced cell death. Once radiation is applied, there is a percentage, S, of non stem cells that die.

$\bar{p_0}$	$\bar{p_1}$	$\bar{\lambda}$	$\bar{d_o}$	$\bar{d_i}$	d	g_0	g_1	g_2	v_1	a
1	0.1	0.005	0.08	0.355	0.067	0.00005	0.0035	1	$\frac{3.9}{7}$	4

Table 13: The parameters above were used to successfully reach the experimental data as seen on figures 11-13 when 4 Gy radiation is introduced.

	Numerical	Experimental	Std. Deviation
x_{os}	0	0	0
x_{is}	652.4	658.2	30.9
Total Population	262304.9	263106.3	8477

Table 14: This table represents the results at day 6 for the case where the are no original stem cells present.

	Numerical	Experimental	Std. Deviation
x_{os}	184.8	147	7
x_{is}	555.6	470.9	140.7
Total Population	262012.2	263106.3	8477

Table 15: This table represents the results at day 6 for the case where the original stem cell count is at 2%.

	Numerical	Experimental	Std. Deviation
x_{os}	924.2	970	70.7
x_{is}	315.6	349.3	35.2
Total Population	260704.8	263106.3	8477

Table 16: This table represents the results at day 6 for the case where the original stem cell count is at 10%.

4.2.2 The Effects of 8 Gy

As in the 4 Gy case, we use the same parameters as we did for the 0Gy radiation case. With the dose of radiation increased, the rate of dedifferentiation also increases. This time, the increase in dedifferentiation is significantly higher than for the 4 Gy case. Large doses of radiation give rise to more induced stem cells, which is not ideal for a stable biological system. This poses an interesting and challenging biological problem as the number of SCs correlates strongly with tumor recurrence [1]. As we did with 4Gy, the division and direct differentiation rates also drop. One change that was also necessary to occur, was to decrease the gain factor for the original stem cell population. By keeping the gain factor the same, there were an insufficient number of original stem cells. The increase to 8Gy radiation eliminated a large portion of non-stem cells.

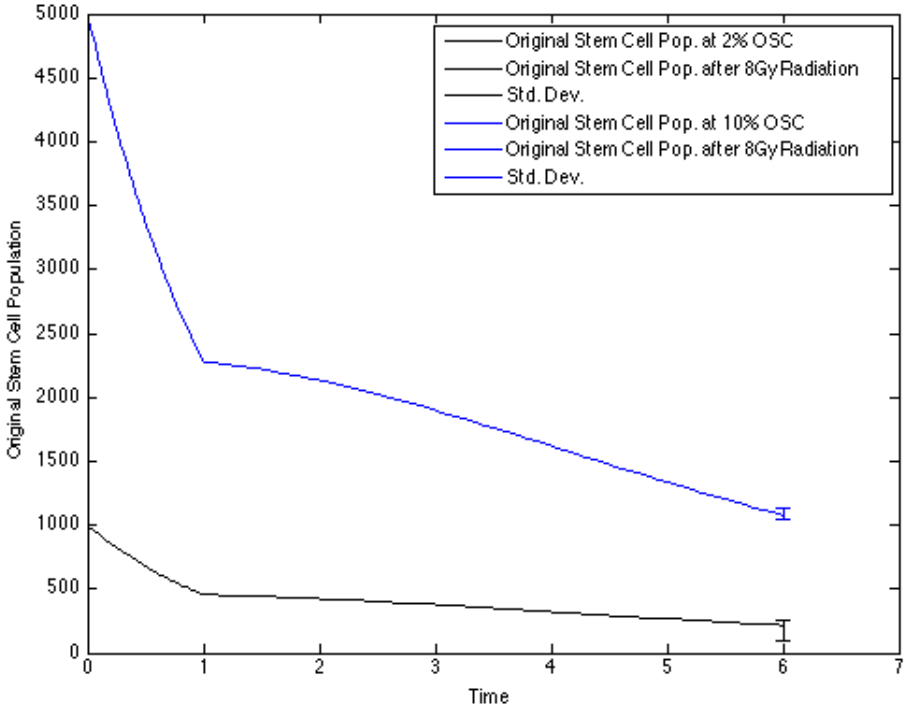

Figure 14: This set of graphs shows the population dynamics of original stem cells with 8 Gy radiation. With 0% original stem cells, there is no increase of population. For the cases of 2% and 10% original stem cells, there is a gradual decrease in the span of six days with a kink at day 1 due to the radiation-induced death of non stem cells.

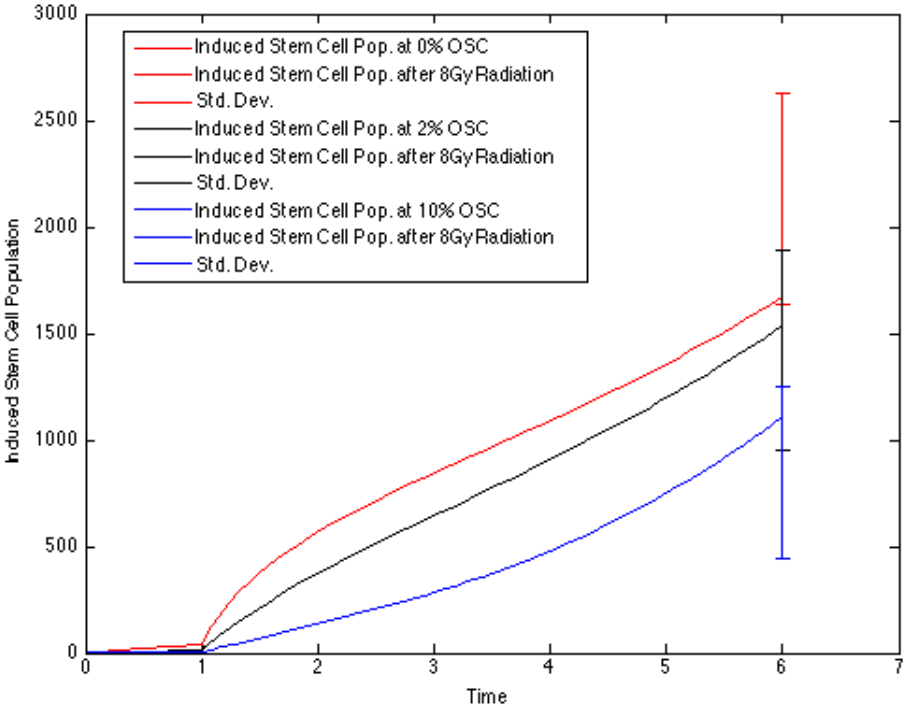

Figure 15: This set of graphs shows the induced stem cell population dynamics with 0%, 2% and 10% original stem cells with 8 Gy radiation. The initial population sizes for all three cases of induced stem cell start at zero. Similar to that of original stem cell population, induced stem cell population also has a kink at t=1 (1 day) after which the induced stem cell populations rapidly increase.

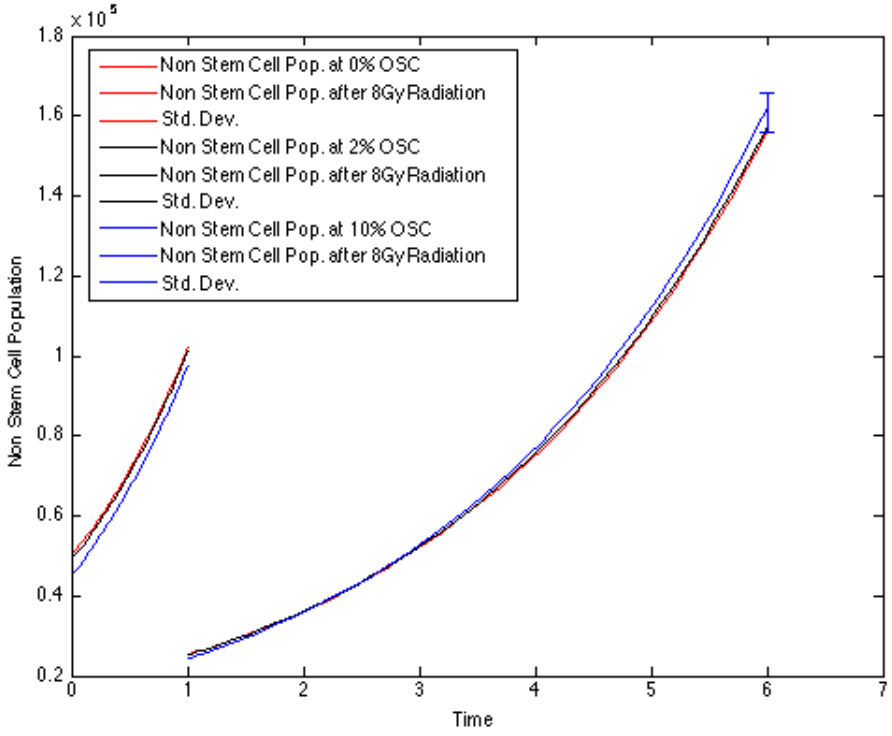

Figure 16: This set of graphs shows the non stem cell population dynamics with 0%, 2%, and 10% of original stem cells. These sets of graphs form a discontinuity due to the radiation applied. Once radiation is applied, there is a percentage S of non stem cells that die.

$\bar{p_0}$	$\bar{p_1}$	$\bar{\lambda}$	$\bar{d_o}$	$\bar{d_i}$	d	g_0	g_1	g_2	v_1	a
1	0.1	0.05	0	0.2	0.03	0.000055	0.0035	1	$\frac{2.8}{7}$	4

Table 17: The parameters above were used to successfully reach the experimental data. These values were used to match figures 14-16 when 8 Gy radiation is introduced.

	Numerical	Experimental	Std. Deviation
x_{os}	0	0	0
x_{is}	1668.6	2133.6	495
Total Population	157928.2	160781.6	4849.8

Table 18: This table represents the values at day 6 for the case where the original stem cell count is at 0.

	Numerical	Experimental	Std. Deviation
x_{os}	215.9	177	75
x_{is}	1536.1	1418.2	470.3
Total Population	159357.8	160781.6	4849.8

Table 19: This table represents the values at day 6 for the case where the original stem cell count is at 2%

	Numerical	Experimental	Std. Deviation
x_{os}	1077.8	1088	39.6
x_{is}	1107.7	847.7	402.7
Total Population	164112.4	160781.6	4849.8

Table 20: This table represents the values at day 6 for the case where the original stem cell count is at 10%

5 Conclusion

We constructed and modified our mathematical model to find effects of radiotherapy on population dynamics. The experimental data we compare the solutions to our math model with, shows a larger induced stem cell population when the dose of radiation is stronger. Why would radiation cause an increase in induced stem cell population, is still an unanswered question. However, in response to the increase in population, our work shows us that the dedifferentiation rate increases with stronger doses of radiation. Our findings suggest that the direct differentiation rate from original stem cells to non-stem cells, $\bar{d}_0$, must approach 0 at a certain time, because were it constant would lead to the depletion of OSC's, thus resulting in a trivial steady state. We have seen that differentiation can occur without division (direct differentiation) from original to non-stem cells. Our findings also suggest that non-stem proliferation rates decrease in response to radiation stress, due to the 10% stem cell culture's total population exhibiting a sudden drop from 425,000 to 263,000, corroborated by evidence from [1]. Cells are most sensitive to radiation during the S phase of the cell cycle, which explains the reduced proliferation rate of non-stem cells [2]. For future work, various radiotherapy treatment cycles, at alternating intensities and intervals can be researched to find optimal treatment methods. We can use our model, or an improved version to calculate the sort of treatment regimen that may effectively minimize the dedifferentiation rate of non-stem cells to prevent complications.

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