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MATHEMATICAL MODELING OF STEM CELL PROLIFERATION

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Abstract.
The mathematical models prevalently used to represent stem cell proliferation do not have the level of accuracy that might be desired. The hyperbolastic growth models promise a greater degree of precision in representing data of stem cell proliferation. The hyperbolastic growth model H3 is applied to experimental data in both embryonic stem cells and adult mesenchymal stem cells. In the embryonic stem cells the results are compared with other popular models, including the Deasy model, which is used prevalently for stem cell growth. In the case of modelling adult mesenchymal stem cells, H3 is also successfully applied to describe the proliferative index. We demonstrated that H3 can accurately represent the dynamics of stem cell proliferation for both embryonic and adult mesenchymnal stem cells. We also recognize the importance of additional factors, such as cytokines, in determining the rate of growth. We propose the question of how to extend H3 to a multivariable model that can include the influence of growth factors.

Keywords: Embryonic stem cells, adult stem cells, hyperbolatic growth models, proliferative index, cytokines

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Introduction

Stem cell research is an area of much current interest, and within this field there is a need for an accurate mathematical model to represent the growth dynamics of stem cells. The purpose of this paper is to present the hyperbolastic growth models of Tabatabai *et al*. [1] as an accurate and effective means of representing the dynamics of stem cell growth. Using experimental data we demonstrate the accuracy and effectiveness of these models, including both adult stem cells and embryonic stem cells.

Within each organism, a source is required for the new cells which are needed on an ongoing basis to replace older cells, to maintain tissue homeostasis, and to respond to environmental stresses. Adult stem cells in each organ are working to fulfill this important role of new cell production. Through its special nature and its niche in the organism, the stem cell is able to self-renew, or to undergo ongoing asymmetric cellular divisions in which the cell neither ages nor loses its special characteristics as a stem cell. In these asymmetric cell divisions, the daughter cells produced from the stem cells will differentiate into one of any number of organ specific cell types. In the process of differentiation toward specific cell types, these daughter cells lose the capacity for self-renewal. Through a combination of the processes of self-renewal, differentiation, and proliferation, all of the cell types needed in the tissue system are produced as daughter cells of the stem cells, while the stem cells themselves are maintained in an nonageing state.

These processes of self-renewal, differentiation, and proliferation are fundamental in the functioning of stem cells. Lie and Xie [2] have described a delicate balance between stem cell self-renewal and differentiation, and the understanding of this point is a basis for understanding of how stem cells regulate the body, their role in tumor growth and formation, and their

therapeutic use in treating human disease. Scientists were led to the concept of stem cell niche by observing the role of the surrounding environment in regulation of stem cell function and determination of the course of their development. The concept of niche refers to the microenvironment for the adult stem cells and a more specific understanding of niche includes a thorough description of cell to cell interactions and extracellular signaling. The stem cell niche is the means by which the body interacts with the stem cells to determine development, maintaining a state of quiescence and self-renewal under normal conditions, but stimulating proliferation and differentiation when additional cells are required by the body, such as times of external stress. Although the concept of niche is derived from the microenvironment of the stem cell within the organism, scientists are attempting to reproduce these conditions *in vitro*, and the concept of niche also extends to regulation of stem cells in this environment.

Much interest has also developed in embryonic stem cells, a type of stem cell from earlier in human development, capable of differentiating into any cell type within the body, from any of the three germ layers. This pluripotency is the reason for interest in embryonic stem cells, and this potential to produce all types of cells in the body carries much potential for both research and regenerative medicine. Adult stem cells are no longer pluripotent, but still maintain the multipotency needed to produce a wide range of cells. Both embryonic and adult stem cells share the properties of self-renewal, differentiation, and proliferation which characterize stem cells; however there are some important differences. The main difference is in differentiation, pluripotency versus multipotency. Although embryonic stem cells may be grown effectively outside the body, adult stem cells have shown resistance to production in large numbers. However adult cells from a patient's body do not risk potential immune rejection as embryonic stem cells do.

All of the above issues of self-renewal, differentiation, and proliferation are critical areas within the field of stem cell research. We focus on presenting an accurate mathematical model for stem cell proliferation, joining other researchers who have already studied this issue. For many stem cell therapies, the efficacy of the treatment will depend on the number of stem cells available for transfer, as discussed in Henrigou *et al*. [3] and Bonab *et al*. [4]. For these reasons a mathematical model representing the size and rate of growth of a population of stem cells will be highly useful. The choice of an accurate growth model is an integral part of the analysis of the growth and will eventually aide researchers in attaining a better understanding of the progression and regression of the population size and the associated rates of change (first and second derivatives) of these growth rates.

We begin with a review of some existing growth models applied to the field of stem cell proliferation. An early model using systems of differential equations was proposed by Loeffler and Wichman [5] to model the regulation of hematopoiesis. Cowan and Morris [6] introduced a model determined using two parameters, representing number of proliferative daughters and rate per proliferative cell per day, from experimental data. Similar in concept is the growth model proposed by Sherley *et al*. [7] to describe the generation of both dividing and non-dividing cells. The Sherley model has the form

$$P(t) = P_0[0.5 + \frac{1-(2\alpha)^{\frac{t}{DT}+1}}{2(1-2\alpha)}] \qquad (1)$$

where $P(t)$ is the population size at time t, P_0 is the initial number of cells, α is the mitotic fraction, and DT is the division time. As the parameter α represents the percentage of the cells for which cellular divisions are ongoing, α must satisfy $0 \leq \alpha \leq 1$. Deasy *et al*. [8] applied the Sherley model to describe the mechanisms of muscle stem cell expansion with cytokines. Jankowski *et al*. [9] used this model to investigate the role of CD34 expression and cellular

fusion in the regression capacity of cells. Deasy *et al*. [10] expanded the Sherley growth model by incorporating a term into equation (1) to account for cell loss and cell differentiation. Their growth model has a form

$$P(t) = P_0[0.5 + \frac{1-(2\alpha)^{\frac{t}{DT}+1}}{2(1-2\alpha)}]\text{-M} \qquad (2)$$

where M is added to take into account the cell loss and one may consider $P(t)$ as the sum of two terms, one corresponds to proliferating cells and the other is associated with differentiated cells. In this paper, we refer to formula (2) as the Deasy growth model.

Methods

The Hyperbolastic Growth Model of Type III (H3)

The analysis of stem cell proliferation is done using the hyperbolastic model H3. The hyperbolastic growth models of [1] were recently introduced in order to provide growth models with more flexibility in the growth rate as the population reaches its carrying capacity, and thus also a greater degree of accuracy. These models have been demonstrated to be highly accurate, particularly in cases of modeling biological growth, as in [1, 11, 12]. For this paper we focus on H3, the hyperbolastic growth model of type III, which we now present.

The growth rate will be given by the nonlinear differential equation

$$\frac{dP(t)}{dt} = (L - P(t))\left(\delta\gamma t^{\gamma-1} + \frac{\theta}{\sqrt{1+\theta^2 t^2}}\right), \qquad (3)$$

with the initial condition $P(t_0) = P_0$, where L, δ, γ and θ are parameters. We refer to the model (3) as the hyperbolastic ordinary differential equation of type III or H3. This rate of

growth is a product of one factor representing the distance of the current population from its limiting value and a second factor including the intrinsic rate δ, an allometric constant γ, and an additional term θ allowing flexibility in growth rate over time. The solution to the equation (3) is the function

$$P(t) = L - \alpha\, EXP\left[-\delta\, t^{\gamma} - \operatorname{arcsinh}(\theta t)\right], \qquad (4)$$

where

$$\alpha = (L - P_0) EXP\left[\delta\, t_0^{\gamma} + \operatorname{arcsinh}(\theta t_0)\right].$$

We call the function $P(t)$ of equation (4) the hyperbolastic growth model of type III or simply H3. If necessary, one can introduce shift or delay parameters in this model. The doubling time t for the model H3, in the case where $P_0 < L/2,$ is the solution to the equation

$$\ln\frac{L-2P_0}{\alpha} + \delta\, t^{\gamma} + \operatorname{arcsinh}(\theta t) = 0.$$

Here we briefly address the biological meaning associated to the parameters L, δ, γ, and θ. The parameter L has the same units as $P(t)$, in this case the number of stem cells, and it represents the limiting value of the size of the population, or the carrying capacity. The parameter δ corresponds to the intrinsic biological growth rate; however the overall rate of growth is jointly determined by all of the parameters δ, γ, and θ. The units of δ is 1/(time)$^{\gamma}$, which in the cases of this paper is 1/(days)$^{\gamma}$. The parameter γ is known as the allometric constant, and a similar parameter occurs in the Weibull model. It is also sometimes called a statistical shape parameter. This parameter is dimensionless. Finally, the parameter θ has units 1/(time), in our case 1/(days), and to more fully describe its biological meaning we rewrite the equation (4) in the form

$$P(t) = L - \frac{\alpha}{\theta t + \sqrt{1+(\theta t)^2}} EXP\left[-\delta t^{\gamma}\right].$$

For θ=0, the term in front of the exponential reduces to α, and the model reduces to the Weibull growth model. When θ≠0, the expression $\alpha(t,\theta) = \frac{\alpha}{\theta t + \sqrt{1+(\theta t)^2}}$ allows this factor to vary with time t, according to this formula and the value of θ. Thus the parameter θ provides variation in the quantity α, which represents a normalization of the distance between the initial population and the limiting value, allowing adjustment of this growth rate over time. In application of the model a constant value is determined for θ that best represents the growth observed in the data.

The hyperbolastic ordinary differential equation of type III can also be represented in the following form

$$\frac{dP(t)}{dt} = a(t) - b(t)$$

for terms $a(t)$ representing factors contributing to population growth and $b(t)$ representing factors slowing or retarding population growth. Here

$$a(t) = L\left(\delta\gamma t^{\gamma-1} + \frac{\theta}{\sqrt{1+\theta^2 t^2}}\right)$$

and

$$b(t) = P(t)\left(\delta\gamma t^{\gamma-1} + \frac{\theta}{\sqrt{1+\theta^2 t^2}}\right).$$

Clearly, as $P(t)$ approaches L, the factors slowing population growth catch up with those increasing population growth, and the overall rate slows to zero. Thus the parameter L gives the carrying capacity, or the level at which the population reaches a plateau. For the intrinsic growth rate, there are the three parameters of δ, γ, and θ, which jointly determine this rate. In the case

where $\gamma=1$ and $\theta=0$, then the intrinsic growth rate is given by δ. However, values of $\gamma>1$ can be used to speed up the time course, thus altering the rate, while values of $0<\gamma<1$ slow the time course. Furthermore the variable θ represents the lack of symmetry inherent in biological growth, with values of θ farther from 0 being farther from the symmetric sigmoidal curve.

The growth rate $P'(t)$ or $\frac{dP}{dt}$ will also be called the velocity of the growth of the cell population. Its units are (cells) / (day). Notice its absolute value equals the speed at which the cell population is growing or receding. Its rate of change, $P''(t)$ or $\frac{d^2P}{dt^2}$ will be called the acceleration of the population growth. The units for $P''(t)$ are (cells) / (day^2). In the subsection on growth of adult mesenchymal stem cells, where $P(t)$ is used to represent the proliferative index (PI), the term velocity of $P(t)$ will represent the rate of change of the proliferative index, and the acceleration of $P(t)$ will be its rate of change. For $P(t)$ measuring the PI, $P'(t)$ has units of 1 / (days), while $P''(t)$ has units of 1 / (days^2).

The parameters are estimated using computational software SPSS and Mathematica to produce a best fit to the experimental data. It is also possible to use the SAS package. The method of non-linear least squares regression for the H3 model (4) is used to determine the model parameters. Using SPSS, the input data can be analyzed using the Nonlinear Regression module, found under Analyze and Regression. After entering formula (4) into the box for Model Expression, it is then necessary to enter initial value estimates for the parameters. In SPSS, the arcsinh(x) function must be entered using its definition in terms of logarithms: $\operatorname{arcsinh}(x)=\ln(x+\sqrt{1+x^2})$. An example of the source code used to estimate the parameters in SAS can be found in the additional file of [1].

Note that $P'(t)$ and $P''(t)$, the velocity and acceleration of the growth rate, can be explicitly determined, as functions of time, once the parameters for $P(t)$ have been determined. Mathematica is an effective tool for computation of $P'(t)$ and $P''(t)$, as well as for their use in studying the stem cell growth dynamics. Description of rate of growth as an explicit function $P'(t)$ is more accurate and realistic than use of a static parameter, for instance. The explicit functions and $P''(t)$ allow for a deeper analysis of the growth dynamics.

Results

Analysis of Embryonic Stem Cell Growth Data.

Previous work of Bursac *et al*. [12] has already demonstrated the effectiveness of H3 in modeling the proliferation of embryonic stem cells. In that paper H3 was shown to represent the data more accurately than other sigmoidal models, such as Weibull, Gompertz, logistic, and Richards. For these other models the Mean Absolute Relative Error ranged from almost ten to over twenty times that of H3. The Deasy and Sherley models, which have been prevalently used in modeling stem cell growth, are of a different type of model, closer to exponential growth rather than of sigmoidal type. As the Deasy model is the more developed and more accurate of these models, we compare this model with the others in the analysis of the embryonic stem cell data. In particular, we compare Deasy and H3 for accuracy in representing the experimental data. The NIH stem cell data [13] is available online. The estimated values of the parameters for the Deasy model are given in Table 1, as computed from the data. The H3 parameter estimates are given in Table 2. Note that the parameter values given in [12] for the H3 model were mistyped, and the correct values are those appearing in Table 2. Table 3 gives the estimated values of the number of stem cells for each of these models, as compared to the observed data.

Table 1. Parameter estimates for the Deasy model using embryonic stem cell data.

Parameter	Estimate	Std. Error
α	1.000	1.114
DT	2.021	2.338
M	80.670	71.897

Table 2. Parameter estimates for the hyperbolastic H3 model using embryonic stem cell data

Parameter	Estimate	Std. Error
δ	3.137E-6	0.000
L	762.922	3.254
θ	0.051	0.003
γ	7.990	0.248

Table 3. Observed and estimated number of embryonic stem cells (in units of thousands)

Day	Observed Number of Stem Cells	Hyperbolastic H3 Estimated Number of Stem Cells	Deasy Estimated Number of Stem Cells	Absolute Relative Error Hyperbolastic H3	Absolute Relative Error Deasy
1.0	110.000	110.000	74.3343	0.000	0.324
2.0	139.375	142.862	137.751	0.023	0.012
3.0	186.875	184.876	227.114	0.013	0.216
4.0	303.750	304.918	353.037	0.002	0.162
5.0	603.125	603.246	530.480	0.000	0.120
6.0	760.000	760.062	780.520	0.000	0.027

In analyzing the accuracy of these models, the performance of the Deasy model was not good, with a Mean Absolute Relative Error of almost twenty-two times that of H3. In the comparative study, this places it as the least accurate of all the models considered, with even larger errors than the classical sigmoidal models, which do not perform as well as H3. The values for Mean Absolute Relative Error are given by 0.0066 for H3, 0.043 for hyperbolastic H2, 0.063 for Weibull, 0.073 for hyperbolastic H1, 0.121 for Gompertz, 0.131 for logistic, and 0.134 for Richards, and 0.144 for Deasy. See the pie graph, Figure 1, comparing the Mean Absolute

Relative Error for all of these models. The Mean Squares Error for the Deasy model is 3674.616 compared to 8.47 for the hyperbolastic H3. This translates into an almost 434 to 1 ratio of the Mean Squares Error. In order to compare models with different numbers of parameters on an equal basis, the Akaike Information Criterion (AIC) is commonly used [14]. The model with the lowest value of AIC is considered the best. In comparing the performance of the H3 and Deasy models, we have computed AIC for Deasy to be 61.8469 while the AIC for H3 is 24.9781, showing a superior fit. The R^2 for hyperbolastic H3 is 1.000, while the Deasy has R^2 of 0.970.

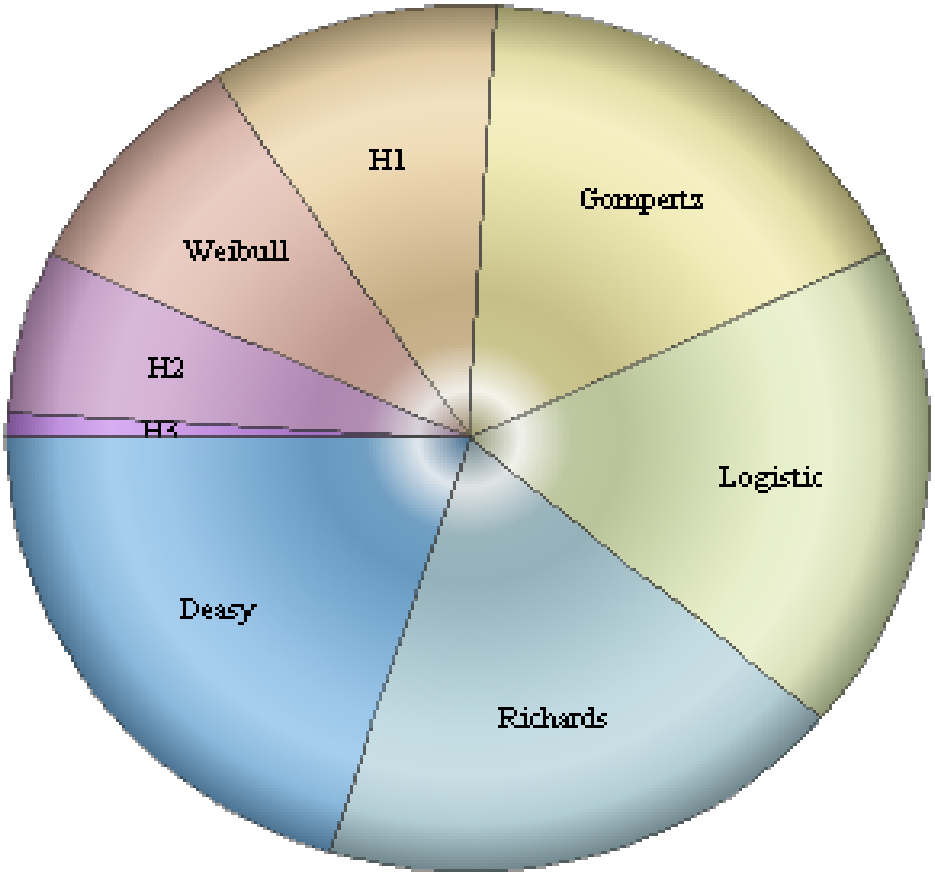

Figure 1. Pie chart showing Mean Absolute Relative Error for all models

Furthermore, the estimates given by the Deasy model vary considerably, between underestimates and overestimates, so that the overall shape is not representative of the data, as can be seen in the graph of Figure 3. This should be compared with the hyperbolastic model H3, as shown in Figure 2, for which there is no visible difference with the observed values. This wider variation is due to the exponential nature of the Deasy model, in contrast to the shape of the data in which the growth rate slows by days 5 and 6. Clearly this data can be better represented by the appropriate sigmoidal model, in particular H3.

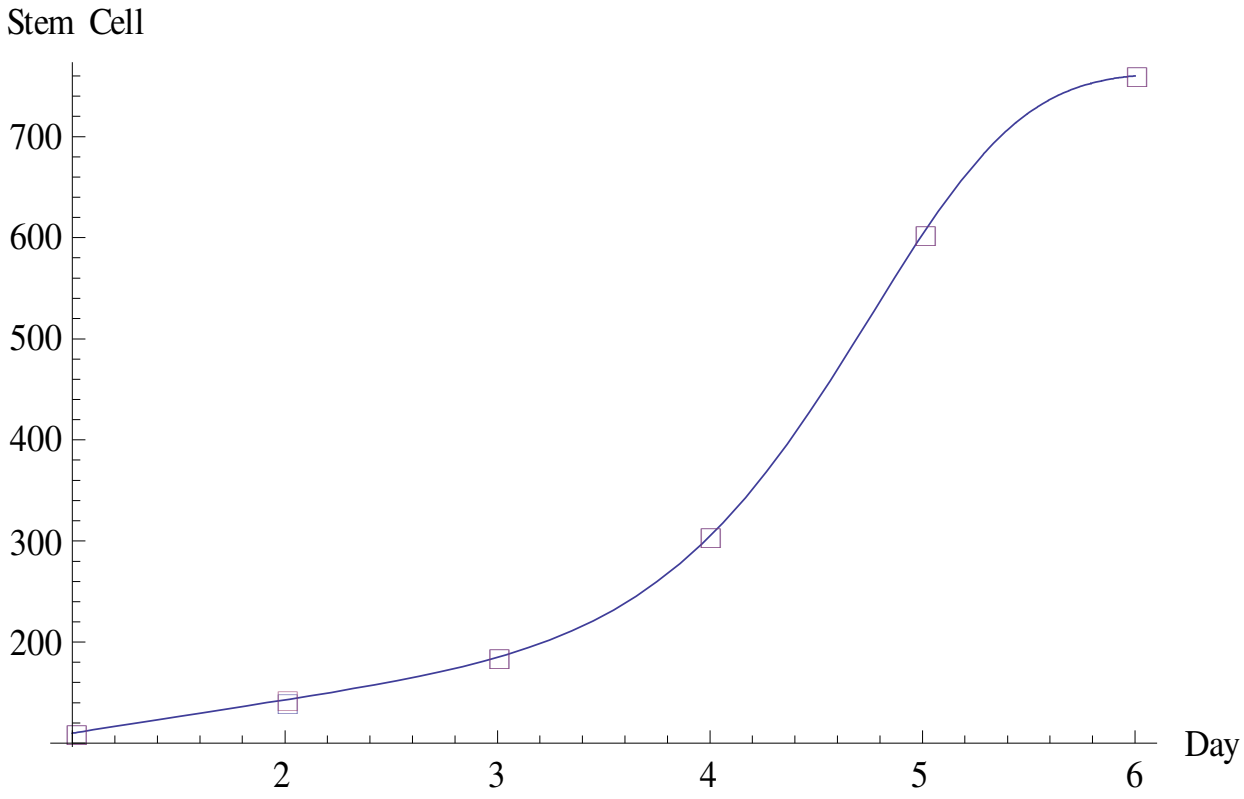

Figure 2. Scatter Plot of Observed and Estimated Number of Embryonic Stem Cells Using Hyperbolastic H3 Model
square represents observed number of embryonic stem cells
circle represents estimated number of embryonic stem cells using H3

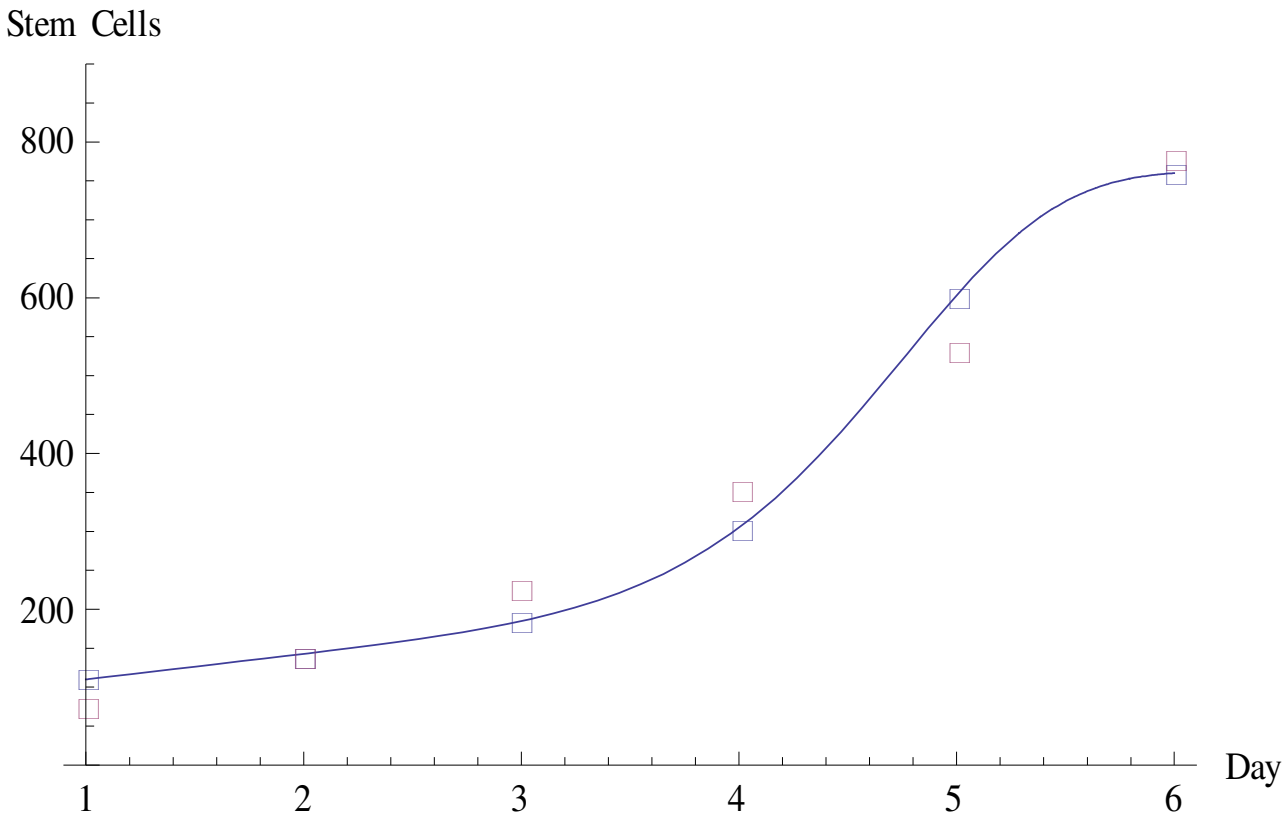

Figure 3. Scatter Plot of Observed and Estimated Number of Embryonic Stem Cells Using the Deasy Model
circle represents observed number of embryonic stem cells
square represents Deasy-estimate of number of embryonic stem cells
Solid curve represents H3-estimate of number of embryonic stem cells

The predictive function and its associated velocity and acceleration are determined from H3 and can be used to analyze the dynamics of the growth. Because of the exactness of the fit to the data points, the derivative of this predictive function at each time gives the instantaneous rate of growth. The model determines that the maximum growth rate of the number of embryonic stem cells is 338.922 thousand per day, and it occurs on the day 4.75982. Based on the comparison above and the fit of the data points, this evaluation of maximum growth rate is more accurate than that predicted by the Deasy model or other models. Deasy estimates a maximum rate of 295.364 on day 6, and will always estimate the last day as the highest rate of growth. In actuality the growth has leveled off by this point, and hyperbolastic estimates it as 19.9461 thousand cells

per day. Furthermore on day 4.75982, at the maximum rate, the growth rate is estimated by Deasy as 193.033, considerably underestimating the actual value.

The mitotic fraction and quiescent rate of the proliferating cells, represented in the Deasy model by α and 1- α, are varying over time, or possibly with respect to changes in other variables, in a realistic biological setting. Similarly the dividing time, represented by DT in the Deasy and Sherley models, can also vary. This is the reason for the distance between the predicted and observed values for these models. However, it is possible to make an experimental measurement of the rate of proliferation in terms of the mitotic fraction, by counting the numbers and percentages of cells observed to be in the various stages of the cell cycle. This measurement is known as the Proliferative Index (PI), and it is used to measure the proliferation of adult mesenchymal stem cells in the following section.

Modeling Proliferative Index of Mesenchymal Stem Cells.

Adult stem cells active in a niche within a living system must maintain a proper balance between self-renewal, differentiation, and proliferation. This section analyzes the data from the study of Wang *et al*. [15] linking a decreased proliferative rate for mesenchymal stem cells to osteonecrosis of the femoral head, a pathology in which bone mass in the head of the femur decays while bone tissue dies, often requiring hip replacement surgery. These authors believe that the decreased proliferation of the mesenchymal stem cells is induced by prolonged treatment with corticosteroids and that this decreased proliferation leaves a shortage of the cells required for homeostasis and repair of the bone tissue. It has been suggested that decreased proliferation of mesenchymal stem cells is the cause of osteonecrosis, and several studies of Gangil *et al*. [16, 17] demonstrated that therapy with hematopoietic and mesenchymal stem cells through bone

marrow transplant can have a positive impact in the outcome, particularly for patients treated in the early stages. Another study [3] gives similar results, and, by counting the number of progenitor cells transplanted, they are also able to conclude that transplantation of larger numbers of progenitor cells leads to better outcomes. The data analyzed in this section [15] also provide support to the role of mesenchymal stem cells in osteonecrosis of the femoral head. These authors studied bone marrow from a group with osteonecrosis and for a control group of healthy individuals, and the rates of proliferation of the mesenchymal stem cells were compared between these groups. Even though the individuals with corticosteroid-induced osteonecrosis were considerably younger, their mesenchymal stem cells displayed a significantly decreased level of proliferation, as compared to the control group.

In the data we analyze from Wang *et al*. [15], the proliferative rates of mesenchymal stem cells are compared between a control population and a population with corticosteroid-induced osteonecrosis of the femoral head. The patients with osteonecrosis were considerably younger, with a mean age of 29.7 years, as compared to 60 years. In order to measure proliferation 3 to 5 mL of bone marrow was obtained from the femoral head, and mesenchymal stem cells were isolated for study. The rate of proliferation was measured using the MTT reduction assay method. The proliferative index

$$PI = \left[\left(S + G_2 / M \right) / \left(G_0 / G_1 + S + G_2 / M \right) \right] \times 100\%$$

was used to measure the proliferation of the stem cells, rather than a strict count of the cells. This means of determining proliferation is often more practical on an experimental basis, as compared to a direct count of all the cells. The proliferative index represents the percentage of cells which are in stages S and G_2/M of the cell cycle. This percentage represents proliferation because it gives the percentage of cells in the cell cycle at a point near to proliferation, from the

synthesis of DNA onward. The cells in stage G_0 are quiescent, while the time of the stage G_1 is the primary determinant of the length of the cell cycle. Excluded from the percentage measuring proliferation are those cells in the quiescent stage of G_0 or the first gap phase G_1. We use H3 to model the proliferative index for the mesenchymal stem cells obtained from the bone marrow for both the osteonecrosis group and the control group. The models H1 and H2 of [1] would also be available as effective models. Because the proliferative index represents the percentage of cells in proliferative stages of the cell cycle, the Sherley and Deasy models will not apply. The parameter estimates produced in fitting H3 to the experimental data are given in Table 4. Table 5 presents the experimental data [14], together with the estimated values given by H3.

	Control		Necrosis	
Parameter	Estimate	Std. Error	Estimate	Std. Error
δ	3.250E-6	0.000	2.298E-5	0.000
L	0.840	0.006	0.815	1.136
θ	0.113	0.004	0.046	0.095
γ	6.628	0.545	4.615	3.500

Table 4. Parameter values for H3 for adult mesenchymal stem cells

Day	Observed PI, control group	Estimated PI, control group	Observed PI, osteonecrosis group	Estimated PI, osteonecrosis group
0.0	0.1788	0.1788	0.1947	0.1947
1.0	0.2425	0.2493	0.2360	0.2226
3.0	0.3723	0.3682	0.2669	0.2764
5.0	0.5046	0.5044	0.3428	0.3399
7.0	0.7530	0.7526	0.4363	0.4385
9.0	0.8398	0.8397	0.5832	0.5834

Table 5. Observed and estimated Proliferative Index

The hyperbolastic model H3 gives highly accurate estimates for both the control group and the osteonecrosis group. The control group has an R^2 of 1.000, while the osteonecrosis has an R^2 of 0.997. The mean square error for residuals for both the osteonecrosis and the control groups are both nearly zero, 3.163×10^{-5} for the control data and 1.416×10^{-4} for the osteonecrosis data. Note that the exponential nature of the Deasy and Sherley models makes them unsuitable for modeling this type of data.

By estimating the parameters, one can express the proliferative index explicitly as a function of time for both the control data and the osteonecrosis data. In Figure 4 the estimated proliferative index curve of the control data is graphed with a solid curve, and the estimated proliferative index curve of the osteonecrosis data is graphed with a dashed curve, and the observed data points are also represented on the graph. The time course of the cellular proliferation in this data clearly shows the much lower rate of proliferation among mesenchymal stem cells from the patients with osteonecrosis. In the Figure 5 and Figure 6 we use the functional form to analyze the velocity and acceleration of change in the proliferative index for both the control group (Fig. 5) and the osteonecrosis group (Fig. 6).

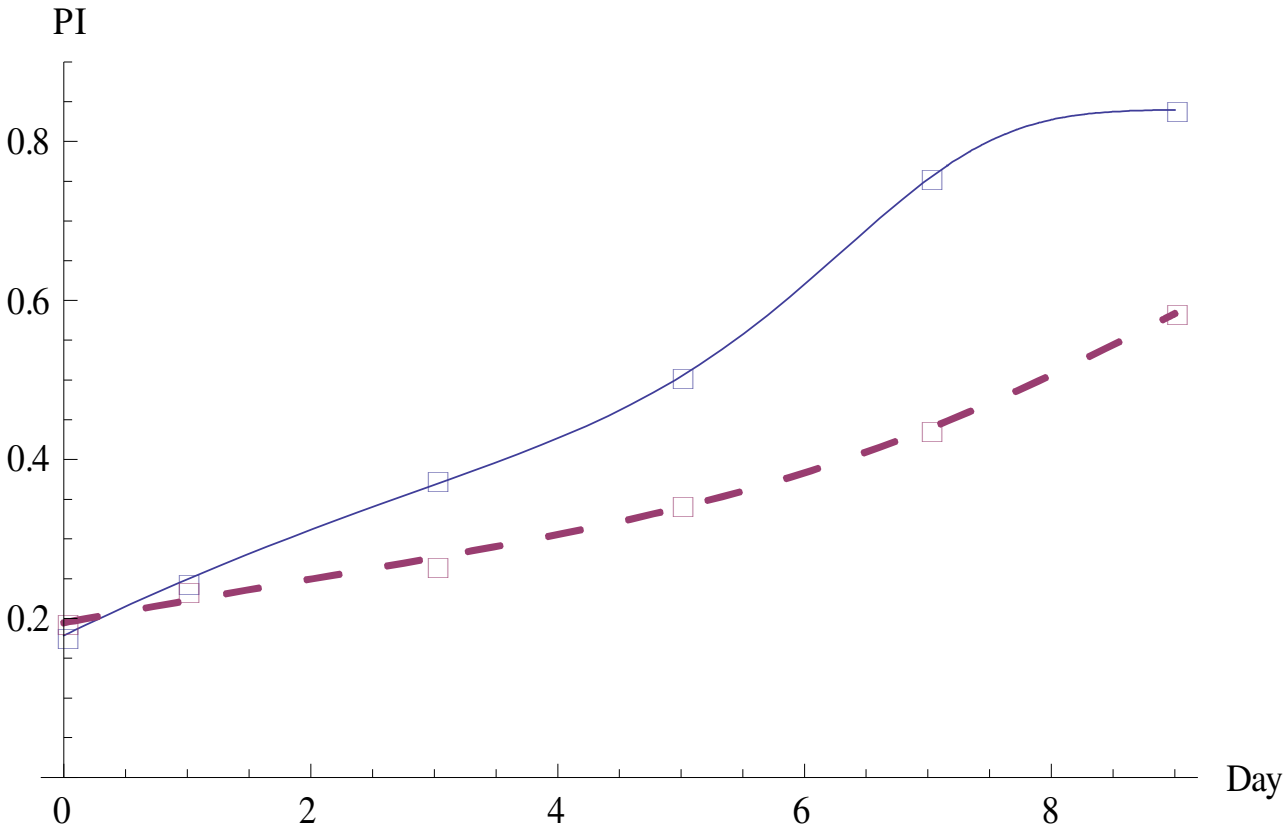

Figure 4. Proliferative Index of MSC, control and osteonecrosis groups
square represents observed values for osteonecrosis group
circle represents observed values for control group

In Figure 5, we see the estimated proliferative index from the control group in the dotted curve, its velocity in the dashed curve, and its acceleration in the solid curve. Here we see a steady increase in the proliferative index from the beginning, with a sustained positive velocity. The velocity does not slow significantly until after day 7.5 when the proliferative index is already well over 80%. The maximum rate of increase of the proliferative index for the control group is 0.13788 on day 6.31435. This maximum rate of increase is achieved when the value of the PI is 0.663201.

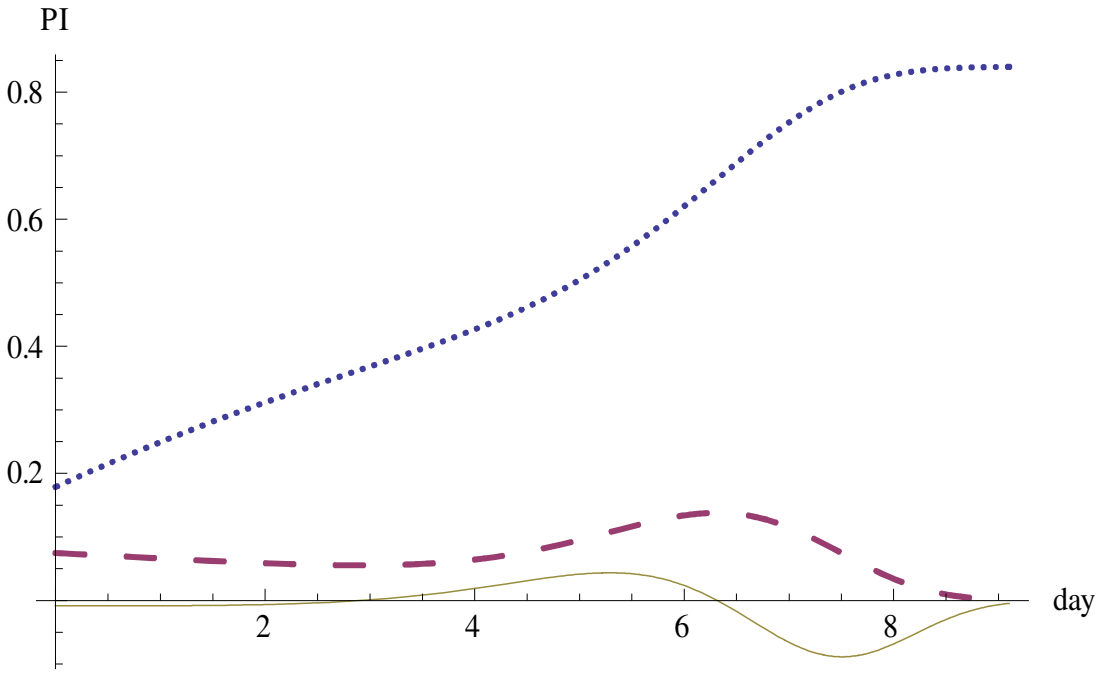

Figure 5. Control group: PI, velocity, and acceleration
dotted is PI, dashed is velocity, and solid is acceleration

In Figure 6, we see the estimated proliferative index from the osteonecrosis group in the dotted curve, its velocity in the dashed curve, and the acceleration in the solid curve. In contrast, for the mesenchymal stem cells from the osteonecrosis patients, the rate of increase of the PI stays at a lower level throughout this time period, although it is increasing near the end and reaches a maximum of 0.0790102 at day 9.06033. This occurs when the PI is 0.588202. The proliferative index is still below 60% after day 9, although it is increasing more at the end. Here the mesenchymal stem cells from the osteonecrosis group have a uniformly lower proliferative index, and the rate of increase of the proliferative index is also significantly lower during the first seven days, often less than half the rate for the control group.

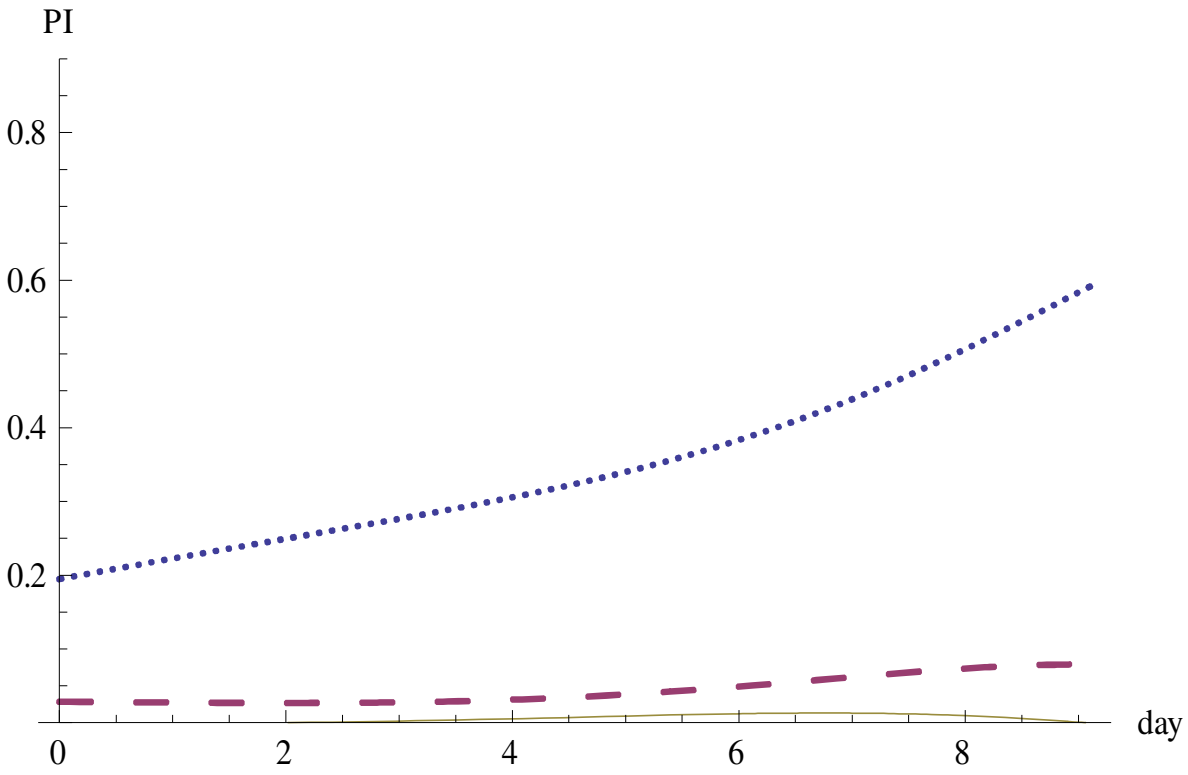

Figure 6. Osteonecrosis group: PI, velocity, and acceleration
dotted is PI, dashed is velocity, and solid is acceleration

The H3 model has been shown to yield highly accurate representation of stem cell growth, both for adult and embryonic stem cells. In the previous section, the accuracy of H3 was verified for embryonic stem cells. In this section its accuracy is also demonstrated for the proliferation of adult mesenchymal stem cells *in vitro*. Furthermore the model applies not only in the case of counting the number of cells, but can also be used to model the proliferation index (PI) representing the rate of proliferation of a group of cells. Expansion of mesenchymal stem cells *ex vivo* promises to be an area of medical importance [18], as therapies require many mesenchymal stem cells, which are only found at very low frequencies in bone marrow. We recommend H3 as an appropriate mathematical model to represent such proliferation of stem cells.

Discussion

In analyzing the growth of embryonic stem cells, H3 is compared with the classical sigmoidal models Weibull, Gompertz, logistic, and Richards in [12], as well as hyperbolastic H1 and H2. In Section 3 we extend the comparison to the Deasy model, one of the prevalent models used for stem cell proliferation. Note that the Deasy model is comparable to the exponential model in the sense that it does not include a carrying capacity, or limiting level of growth, in the assumptions. Thus growth does not slow with an increasing population size. We believe this is one weakness of the Deasy model, particularly for long time frames in which growth must slow. The Deasy model is close in concept to the exponential model but includes a parameter α to represent the mitotic fraction. Here α represents the percentage of cells which continue dividing, while 1-α represents the percentage which have become quiescent. Perhaps this model would display greater accuracy if $\alpha = \alpha(t)$ or $\alpha = \alpha(P,t)$ were allowed to vary with time or with time and population, more closely approximating the biological reality. In this way it would be possible for α(t) to decrease with an increasing cell population, and very likely the resulting model would better fit experimental data. Note that H3, as given in (3), is a non-autonomous differential equation in which the intrinsic rate of growth, jointly determined by δ, γ, and θ, does vary with time. It is furthermore in the tradition of the classical sigmoidal models, so that population also has an effect on determining the overall rate of growth, which slows for higher population size P, as given in (3). This is both the expected result corresponding to the biological theory, and it also corresponds more closely to the biological reality as reflected in experimental data.

We have just observed how the significant difference in accuracy between the H3 and Deasy models for embryonic stem cells is directly related to the self-limiting nature of the growth found in the experimental data. The biological basis behind the self-limiting growth is a fundamental point for those scientists seeking to better understand the proliferation of stem cells. In the case of stem cells, growth may become self-limiting as a consequence of a reduction in the rate of the cells or because of an increase in the rate at which the cells differentiate to form new populations. The slowing rate of proliferation could be caused by factors ranging from limitations in space or resources, to internal programming that tells the cell population it has reached the desired size, or external signaling that helps determine the rate of proliferation. The balance between proliferation and differentiation could similarly be controlled by internal programming of the cells or external signaling. To describe more fully the means of this control is one of the main issues for researchers studying stem cells. Although our model does not directly solve these problems, it can give us important information describing the rate of growth and when the growth becomes self-limiting.

In the study of adult mesenchymal stem cell proliferation, the growth rate was measured using the proliferative index, which is a direct count of the percentage of cells in non-quiescent stages of the cell cycle. The sigmoidal nature of the H3 model, as well as H1 and H2 of [1], allow for modeling of proliferative percentage as a function of time, whereas exponential type models such as Deasy do not apply. In exploring the proliferative percentage of adult mesenchymal stem cells, the H3 model, its parameters, and the associated velocity allow a comparative analysis of cellular proliferation between patients with osteonecrosis and the control group. There is a profound difference in the velocity, or rate of increase of the PI for these cells in the MTT assay. The cells from the control group exhibit a rate of increase approximately twice that of the

osteonecrosis group, reaching a maximum rate on day 6.31435, and reaching a plateau around day 8. Although the limiting value for the control group is only somewhat larger, the osteonecrosis group is significantly delayed in approaching its limiting value. This is revealed in analysis of the velocity $P'(t)$, or rate of change of the proliferative index, which does not reach a maximum until after day 9. All of this analysis of cell proliferation supports the medical and biological analysis of [15], describing reduced proliferative activity of adult mesenchymal stem cells as an important factor in osteonecrosis.

Of the parameters for H3 describing the proliferation of the cells, the value of L directly tells us the limiting value for the cell population, while the parameters δ, γ, and θ jointly determine the rate of growth and the means by which the population approaches the limiting value, as described in the Methods section. The parameter L, the limiting value for the population, holds the key information about the size the population will reach, and scientists can investigate what additional factors may alter the magnitude of L. For the issue of the self-limiting growth, it turns out the inflection point of the curve is the key point. This point is where the fastest rate of growth occurs and where the growth changes from self-accelerating to self-limiting. Thus it is highly significant that the inflection point in H3 is more flexible than it is in other sigmoidal models. In the embryonic stem cell proliferation data, we used $P'(t)$ and $P''(t)$ to determine this maximum rate of growth of 339.922 thousand cells per day on day 4.75982. In [1], the location of the inflection point is given by the time $t = t_0$ that solves of the equation

$$\left[\delta\gamma {t_0}^{\gamma-1} + \frac{\theta}{\sqrt{1+\theta^2 {t_0}^2}}\right]^2 = \left[\delta\gamma\left(\gamma-1\right){t_0}^{\gamma-2} - \frac{\theta^3 t_0}{\left(1+\theta^2 {t_0}^2\right)^{3/2}}\right]. \qquad (5)$$

In the case where θ=0, the solution reduces to $t_0 = \sqrt[\gamma]{\frac{\gamma-1}{\gamma}\cdot\frac{1}{\delta}}$, demonstrating the dependence of the location of the inflection point on the parameters δ and γ in this reduced case. More generally the dependence of this point of transition from self-accelerating to self-limiting growth is more complicated, but it can still be determined from the equation (5) using numerical means. Furthermore, using all the parameters to define $P'(t)$ explicitly, it is possible to investigate the dynamics of the cellular proliferation and the transition to self-limiting growth. Especially when investigating the role of additional factors in influencing the growth rate, it is helpful to see when and how the proliferation transitions from self-accelerating to self-limiting and what is the role of these additional factors.

Tight control and regulation of stem cell function is required within the body in order to achieve the proper function, and this control takes place through the stem cell niche. Researchers investigating the means of this control have focused on the role of cytokines in the local environment and the interaction with the internal programming of the stem cells [2]. Scientists have furthermore tried to reproduce a similar control of the proliferation, differentiation and self-renewal of stem cells outside the body. Watt and Hogan [19] suggest that approaching the full potential of stem cells to treat degenerative diseases will require a more thorough understanding of the regulation of stem cells through signals within the niche. An important goal in stem cell research is to sufficiently understand the signaling and means of regulation within a given stem cell niche to gain control of the stem cells and their growth outside the body. Achieving this goal would be comparable to establishing an *ex vivo* stem cell niche.

As much of the control of stem cell growth is believed to take place through cytokines, scientists have been actively researching the effects of cytokines on stem cell proliferation and

differentiation. See, for instances, the papers of Heo *et al.* [20], Eiselleova *et al*. [21], Chung *et al*. [22], and Schuldiner *et al*. [23]. More generally, other conditions such as the level of oxygen, or of nutrients such as glucose, can also affect the growth of stem cells, as demonstrated in works such as [24, 25, 26]. Multivariable versions of the hyperbolastic growth models have been developed and applied in other works, and in a future work we plan to return to explore the issue of a multivariable model for stem cell growth that will aid researchers in measuring the effects of cytokines, oxygen, and other factors on the rate of proliferation. Such a model could be useful in determining the effects of various cytokines on stem cell proliferation and differentiation. Furthermore an accurate model in this area is desirable, and it could help to explore the impacts of individual explanatory variables or relationships between several such variables. In the work of Lemon *et al.* [27], a mathematical model is developed which describes the proliferation and differentiation of mesenchymal stem cells along artificial scaffolds, and the level of oxygen is shown to play a significant role. This model helps to describe the proliferation of cells on artificial materials and can be expected to play an important role in the area of tissue engineering. A multivariable version of the hyperbolastic models for stem cell growth may also have similar applications.

The hyperbolastic growth model H3 is very accurate in predicting the dynamic behavior of stem cells. This model can be used to understand the growth dynamics of cell populations such as cell proliferation and quiescence rates both *in vivo* and *in vitro*. Proliferative index, another important measure of the rate of growth of a cell population that is often considered in scientific data, can also be modeled accurately with the hyperbolastic growth models. For the analysis of stem cell growth of any type, we believe that the hyperbolastic model H3 or its multivariable

counterpart should be considered as a model of choice for representing growth dynamics of stem cells and also be compared with other growth models before a final decision is made.

References

1. Tabatabai M, Williams DK, Bursac Z (2005) Hyperbolastic growth models: theory and application. *Theor. Biol. Med. Model.* 2(1): 1-13. doi: 10.1186/1742-4682-2-14

2. Li L, Xie T (2005) Stem cell niche: structure and function. *Annu. Rev. Cell Dev. Biol.* 21: 605-631.

3. Hernigou P, Beaujean F (2002) Treatment of osteonecrosis with autologous bone marrow grafting. *Clin. Orthop. Relat. Res.* 2002, 405: 14-23.

4. Bonab MM, Alimoghaddam K, Talebian F, Ghaffari SH, Ghavamzadeh A, Nikbin B (2006) Aging of mesenchymal stem cells *in vitro*. *BMC Cell Biol*. 2006, 7: 14. doi: 10.1186/1471-2121/7/14.

5. Loeffler M, Wichman HE (1980) A comprehensive mathematical model of stem cell proliferation which reproduces most of the published experimental results. *Cell Prolif.* 1980, 13(5): 543-561.

6. Cowan R, Morris VB (1987) Cell population dynamics during the differentiative phase of tissue development. *J. Theor. Biol.*, 122(2): 205-224.

7. Sherley JL, Stadler PB, Stadler JS (1995) A quantitative method for the analysis of mammalian cell proliferation in culture in terms of dividing and non-dividing cells. *Cell Prolif,,* 28(3): 137-144.

8. Deasy BM, Qu-Peterson Z, Greenberger JS, Huard J (2002) Mechanisms of muscle stem cell expansion with cytokines. *Stem Cells*, 20(1): 50-60.

9. Jankowski RJ, Deasy BM, Cao B, Gates C, Huard J (2002) The role of CD34 expression and cellular fusion in the regeneration capacity of myogenic progenitor cells. *J. Cell Sci,* 115(Pt 22): 4361-4372.

10. Deasy BM, Jankowski RJ, Payne TR, Cao B, Goff JP, Greenberger JS, Huard J (2003) Modeling stem cell population growth: incorporating terms for proliferative heterogeneity. *Stem Cells*, 21(5): 536-545.

11. Ahmadi H, Mottaghitalab M (2007) Hyperbolastic models as a new powerful tool to describe broiler growth kinetics. *Poult Sci*, 86(11): 2461-2465.

12. Bursac Z, Tabatabai M, Williams DK (2006) Non-linear hyperbolastic growth models and applications in cranofacial and stem cell growth. In: *2005 Proceedings of the American Statistical Association* Biometrics Section [CD-ROM], Alexandria, VA: American Statistical Association; 2006;190-197.

13. NIH data, http://stemcells.nih.gov/research/NIHresearch/scunit/growthcurves.html

14. Akaike H (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, 19(6): 716-723.

15. Wang BL, Sun W, Shi ZC, Lou JN, Zhang NF, Shi SH, Guo WS, Cheng LM, Ye LY, Zhang WJ, Li ZR (2008) Decreased proliferation of mesenchymal stem cells in corticosteroid-induced osteonecrosis of femoral head. *Orthopedics* 2008, 31(5): 444.

16. Gangil V, Hauzeur JP (2005) Treatment of osteonecrosis of the femoral head with implantation of autologous bone-marrow cells. Surgical technique. *J. Bone Joint Surg. Am.*, 87(Suppl 1.1): 106-112.

17. Gangil V, Toungouz M, Hauzeur JP (2005) Stem cell therapy for osteonecrosis of the femoral head. *Expert Opin. Biol. Ther.*, 5(4): 437-442.

18. Väänänen HK (2005) Mesenchymal stem cells. Annals of Med., 37(7): 469-479.

19. Watt FM, Hogan BLM: (2000) Out of eden: stem cells and their niches. *Science*, 287(5457): 1427-1430.

20. Heo JS, Lee YJ, Han HJ (2006) EGF stimulates proliferation of mouse embryonic stem cells: involvement of CA2+ influx and p44/42 MAPKs. Am. J. Physiol. Cell Physiol., 290(1): 123-133. doi:10.1152/ajpcell.00142.2005.

21. Eiselleova L, Matulaka K, Kriz V, Kunova M, Schmidtova Z, Neradil J, Tichy B, Dvorakova D, Pospisilova S, Hampl A, Dvorak P (2009) A complex role for FGF-2 in self-renewal, survival, and adhesion of human embryonic stem cells. *Stem Cells*, 27(8): 1847-1857.

22. Chung BG, Flanagan LA, Rhee SW, Schwartz PH, Lee AP, Monuki ES, Jeon NL (2005) Human neural stem cell growth and differentiation in a gradient-generating microfluidic device. *Lab. Chip.* 5(4): 401-406.

23. Schuldiner M, Yanuka O, Itskovitz-Eldor J, Melton DA, Benvenisty N (2000) Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells. *Proc. Natl. Acad. Sci.* 97(21): 11307-11312.

24. Khoo JLM, McQuade LR, Smith MSR, Lees JG, Sidhu KS, Tuch BE (2005) Growth and differentiation of embryoid bodies derived from human embryonic stem

cells: effect of glucose and basic fibroblast growth factor. *Biol. Reprod.* 73(6): 1147-1156.

25. Narbonne P, Roy R (2006) Regulation of germline stem cell proliferation downstream of nutrient sensing. *Cell Divsion* 1; 29. doi: 10.1186/1747-1028-1-29.

26. Grayson WL, Zhao F, Bunnell B, Ma T (2007) Hypoxia enhances proliferation and tissue formation of human mesenchymal stem cells. Biochem. Biophys. Res. Commun. 358(3): 948-953.

27. G. Lemon, S.L. Waters, F.R. Rose, and J.R. King (2007) Mathematical modeling of human mesenchymal stem cell proliferation and differentiation inside artificial porous scaffolds. J. Theor. Biol., 249(3): 543-553.